GLYCOSURIA

AND ALLIED CONDITIONS

GLYCOSURIA
AND ALLIED CONDITIONS

BY

P. J. CAMMIDGE, M.D. (Lond.)

PREFACE

THE work on diseases of the pancreas which I commenced some
fourteen or fifteen years ago made it necessary for me to familiarise
myself with much that had been written on diabetes and allied
conditions. Since then I have kept in touch with the litera-
ture, and also carried out many researches bearing on these sub-
jects, while an increasing number of cases of pancreatic disease and
diabetes having been sent to me for observation and treatment,
I have had considerable opportunity of extending my clinical know-
ledge of the various types of glycosuria. At the request of several
friends in the profession I have eventually consented to collect in
an accessible form the conclusions I have arrived at, and at the
same time summarised the work of others.

Glycosuria is essentially a chemical problem, and it therefore
seemed to me advisable that it should be attacked from a chemical
standpoint, leading from this to its pathology, symptomatology,
diagnosis, and treatment. I have consequently first dealt with the
tests, differentiation, and quantitative estimation of the reducing
substances met with in the urine, prefacing this by a brief summary
of the chemistry and physiology of the carbohydrates and their
derivatives. For those who are interested in the chemical questions
involved, I have considered this part of the subject more fully in
the Appendix.

The experimental production of glycosuria in animals has natu-
rally received attention before the pathology of human diabetes
has been considered. In this connection I have dealt at consider-
able length with the relation of the ductless glands, and particularly
the pancreas, to glycosuria, but, I hope, not more fully than the
importance of the subject warrants.

Although for convenience' sake the simpler forms of alimentary,
transitory, and intermittent glycosuria have been dealt with
separately, it is important that it should be realised that they pass
by a gradual transition, with no well-defined boundary, into per-
sistent glycosuria, and this in its turn into typical " diabetes."

As yet the treatment of glycosuria is primarily dietetic, and it is
therefore important that the metabolism of the healthy organism,
and the variations that occur in association with the presence of
sugar in the urine should be thoroughly understood, for this reason
they have been written of in some detail. Since in my opinion it
is not enough that a diabetic should be given a list of foods that he
" may take " and " must avoid," I have outlined a system that I
have adopted by which the diet is worked out, not only as regards

GLYCOSURIA

AND ALLIED CONDITIONS

CHAPTER I

CLASSIFICATION, PROPERTIES, AND PHYSIOLOGY OF THE CARBOHYDRATES AND THEIR DERIVATIVES

STRICTLY speaking, the term "glycosuria," or "glucosuria," should be used only to describe the existence of an abnormal amount of glucose or dextrose in the urine, but it has for so long been employed to designate conditions in which an excess of any sugar is met with that it is convenient to retain it as a generic term with that significance, and to employ the names "dextrosuria," "levulosuria," "pentosuria," &c., when speaking of conditions in which dextrose, levulose, pentoses, and other reducing substances are alone present. In recent years German medical writers have made use of the term "mellituria" in speaking of the excretion of sugar in the urine generally. Although it has occasionally been used by English authors in this sense, it has also sometimes been employed as synonymous with saccharosuria, the condition when cane-sugar is present in the urine, and is therefore not without objection.

The accumulated experience of over one hundred years, since Dobson of Liverpool first obtained sugar from diabetic urines, in 1776, has shown that there are few symptoms that are associated with so many distinct and widely different pathological conditions as glycosuria. The discovery that the reducing substance that occurs in the urine is not always dextrose, and may not even be a sugar, made it necessary to revise some of the earlier work that had been done on glycosuria ; but, although it is now certain that a reduction previously ascribed to glucose is in some instances due to the presence of other bodies, the list of conditions in which dextrosuria may occur is still a lengthy one, and includes the following :—

Diseases of the pancreas, liver, thyroid, pituitary gland, adrenals, kidneys ; cholelithiasis, intestinal disorders (enteritis, colitis,

A

corrosive and food poisoning), chyluria, diseases of the nervous system, psychic conditions (worry, shock, mental strain), pregnancy, tumours of the uterus and ovaries, inanition (vagabond's glycos- uria, &c.), asphyxia, cold immersion (attempted drowning), acute fevers (pneumonia, scarlet fever, measles, mumps, variola, malaria, acute rheumatism, phlegmonous diseases), the administration of thyroid extract, adrenalin, and drugs (atropine, morphine, strych- nine, curare, amyl nitrate, copaiba, phosphorus, perchloride of mercury, uranium salts, phloridzin, acetone, chloroform, ether, nitrous ether, &c.), coal-gas, and carbon monoxide, poisoning, and alcoholic excess (especially champagne and beer).

Some of these are exceedingly rare, and have only been re- ported in one or two cases, while others are comparatively common ; but the difficulty arises that glycosuria is not a constant symptom of any one of the conditions mentioned. The problem of the essential cause of glycosuria was still further complicated when it was shown by experiment on animals that sugar may be made to appear in the urine as the result of a variety of different pro- cedures. A discussion of glycosuria and allied conditions covers, therefore, a very wide field.

Before considering in detail the different varieties of glycos- uria, the symptoms with which they are associated and the con- ditions with which they may be confounded, it will be convenient to first deal briefly with the chemistry of the commoner carbo- hydrates, the changes they undergo during digestion and assimila- tion in the body, the means by which the sugars are recognised, differentiated, and estimated in the urine, and the bearing of modern experimental work on the production of glycosuria.

CLASSIFICATION, COMPOSITION, CONFIGURATION, AND PROPERTIES OF THE CARBOHYDRATES

The carbohydrates constitute an ill-defined group of sub- stances differing widely in their properties and constitution, so that it is difficult to give a satisfactory definition. They may be roughly defined as bodies composed of carbon, hydrogen, and oxygen in which the ratio of hydrogen to oxygen is the same as in water. This definition includes, however, bodies such as inosite, lactic acid, &c., which are not regarded as carbohydrates. The name carbohydrate was originally given to the group because its constituents may be represented as if they were composed of carbon and water in different proportions—e.g. $C_6(H_2O)_6$, $C_{12}(H_2O)_{12}$, with a general formula $C_n(H_2O)_n$, in which " n " is a variable quan-

tity, but in reality they are much more complex. The group includes all the principal constituents of plants, except water, and, with fat and albumens, the carbohydrates form the chief substances necessary for animal life.

The carbohydrates are divisible into three main groups :—

1. The simple sugars, or monosaccharides, or saccharoses.
2. The invertible sugars, or disaccharides.
3. The colloidal, non-crystallisable, polysaccharides or polyoses.

The Monosaccharides.—The naturally occurring monosaccharides are colourless, odourless, crystalline substances which, in a pure state, are not hygroscopic, but are easily soluble in water, feebly soluble in alcohol, and insoluble in ether. They diffuse through animal membrane. Their aqueous solutions are neutral in reaction, and have a sweet taste, varying in intensity with the kind of sugar. On being boiled with dilute acids they are not resolved into simpler sugars.
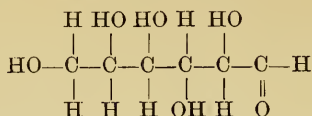
The monosaccharides have the general formula $C_nH_{2n}O_n$, and may be conveniently subdivided, according to the number of carbon atoms they contain, into—

Trioses ($C_3H_6O_3$) containing 3 carbon atoms (*e.g.* glyceric aldehyde)
Tetroses ($C_4H_8O_4$)   ,,   4   ,,   ,,   (*e.g.* erythrose)
Pentoses ($C_5H_{10}O_5$)   ,,   5   ,,   ,,   (*e.g.* arabinose)
Hexoses ($C_6H_{12}O_6$)   ,,   6   ,,   ,,   (*e.g.* glucose)
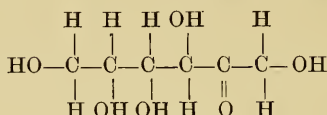Heptoses (7 carbon atoms), octoses (8 carbon atoms), &c.

Many of these sugars have only been prepared artificially, and are merely of theoretical interest. The six, and to a less extent the five, carbon atom sugars are those of chief importance in the animal economy.

The Hexoses.—The hexoses are represented by the molecular formula $C_6H_{12}O_6$. The most important are dextrose (glucose, or grape-sugar), levulose (fructose, or fruit-sugar), galactose, and mannose. Some of the hexoses, such as dextrose and levulose, are found free in nature, or result as hydrolytic decomposition products from the more complex carbohydrates or related nitrogenous substances, the so-called glucosides. For long the hexoses were regarded as consisting of a simple chain of six carbon atoms bound to each other by a single valency, the remaining valencies of five being satisfied by hydrogen and hydroxyl groups, while the sixth was joined to an oxygen atom by a double bond $>$C–O. It was

believed that the carbonyl group ($>C-O$) might be situated at the end of the chain, as, for example, in dextrose—

$$
\begin{array}{ccccccc}
& H & HO & HO & H & HO & \\
& | & | & | & | & | & \\
HO-C & -C & -C & -C & -C & -C & -H \\
& | & | & | & | & | & \| \\
& H & H & H & OH & H & O \\
\end{array}
$$

or might lie between two other carbon atoms, as in levulose—

$$
\begin{array}{ccccccc}
& H & H & H & OH & & H \\
& | & | & | & | & & | \\
HO-C & -C & -C & -C & -C & -C & -OH \\
& | & | & | & | & \| & | \\
& H & OH & OH & H & O & H \\
\end{array}
$$

Sugars which were thought to have the former structure are known as *aldoses*, because they contain the aldehyde group ($-CHO$); those with the latter as *ketoses*, since they contain the divalent ketonic or carbonyl group ($=CO$). The chemical activities of the sugars are not, however, as marked as might be expected from this simple chain formula, and more recently it has been proposed to represent them by a formula in which four of the carbon atoms, together with one oxygen atom, are included in a ring :—
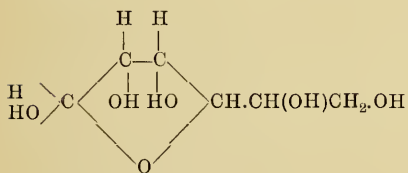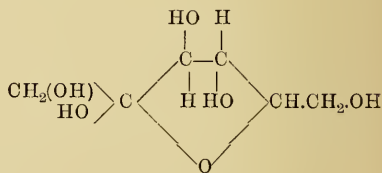
Dextrose   *Levulose*

According to this view the aldehydic and ketonic characters exhibited by the sugars are developed on rupture of the ring by hydrolysis, with the formation of an open chain such as the older formulæ represented. The ring formula has now been very widely accepted, as it is more in accordance with the chemical properties of the sugars. This formula, which would allow of there being sterio-isomeric forms, also accounts for a peculiar physical property of solutions of dextrose and other sugars having aldehyde properties, known as mutorotation or multirotation.

The Pentoses are represented by the molecular formula $C_5H_{10}O_5$. They are widely distributed in the vegetable kingdom as polysaccharides of high molecular weight, the "pentosans," and are never found as simple sugars. They occur in most fruits, particularly in cherries, apples, pears, and plums, and to some extent

in corn and other vegetable tissues. In the animal body pentoses are an important constituent of the nucleo-proteins and nucleic acids, being most abundant in the pancreas. According to Gründ, the percentage of pentose in the dry weight of the pancreas is nearly five times as great as in any other organ of the body (pancreas 2·48 %, liver 0·56 %, thymus 0·56 %, kidney 0·49 %, muscle 0·11 %).

The natural pentoses (arabinose and xylose) are closely related structurally to the natural hexoses. The arrangement of the groups attached to the first four carbon atoms is the same in arabinose as in galactose, and in xylose as in glucose. In this connection it is interesting to note that both xylose and glucose are yielded by some polysaccharides on hydrolysis, and that arabinose and galactose occur together in some gums (Armstrong).

The chemical characters of the monosaccharides are dependent partly upon the hydroxyl groups, and partly upon the carbonyl group, they contain. Through the presence of the hydroxyl groups they are capable of forming esters, or etheral salts, the best known of which is henzoyl-ester, which is sometimes used for their separation and recognition. Owing to the presence of the carbonyl group they are easily oxidised, and so reduce alkaline solutions of the heavy metals. With an ammoniacal silver solution they give a metallic mirror of silver, and reduce alkaline solutions of copper, bismuth, and other metallic salts to oxides and hydroxides of the metal. On this property is based various tests, such as Trommer's, Fehling's, and Böttger's, that are commonly employed for their detection.

The monosaccharide sugars are not precipitated by lead acetate, or sub-acetate, but separate out on making the solution alkaline with ammonia. On being heated they char and form a brown substance, soluble in water, known as caramel. When heated with an alkali, solutions of these sugars turn brown and the sugars are decomposed, forming a variety of substances, including lactic acid, formic acid, and various aldehydes (Moore's test). On being treated with strong acids they break down, yielding furfurol, which can be recognised by the colour reaction it gives with alpha-naphthol, &c. (Molisch's test). The ease with which furfurol is liberated varies with the different sugars, the readiness with which it is evolved from the pentoses forming the basis of several of the more characteristic tests for this group (Phloroglucin test, Orcin test). With phenylhydrazin, these sugars form characteristic compounds which serve to demonstrate their presence in very dilute solutions, and, to a certain extent, to differentiate the various monosaccharides

from each other. Asymmetrical substituted hydrazins of the type $NH_2.NR.C_6H_5$, such as methyl-phenylhydrazin, di-phenylhydrazine, para-brom-phenylhydrazin, also react with the monosaccharides, and, in some cases, form sparingly soluble compounds which are characteristic of a particular sugar, and are therefore of great service in their recognition.

Dextrose and levulose are fermented by yeast, yielding carbon dioxide and alcohol. Galactose is fermented with much greater difficulty, and many varieties of yeast do not act upon it at all. The pentoses are unfermentable, but are attacked and slowly broken down by bacteria.

Like other substances containing an asymmetrical carbon atom, the monosaccharides possess the power of rotating the plane of polarisation of a luminous ray, the exact space relation of the hydroxyl groups relative to the skeleton chain of carbon atoms determining whether the ray shall be deflected to the right or to the left—that is to say, whether sugars with the same gross structure shall be dextro-rotatory or levo-rotatory. The power of rotating polarised light possessed by a particular sugar is, under certain circumstances, a fixed quantity known as its "specific rotation," and, as this property is also exerted by solutions of the sugars, the angle through which rotation occurs serves for their accurate estimation.

The most reliable observations of the specific rotatory power of the monosaccharides in solutions containing about 10 per cent. are as follows :—

Hexoses—d-dextrose	.	.	.	.	.	$+ 52\cdot7°$
d-levulose	.	.	.	.	.	$- 93\cdot8°$
d-galactose	.	.	.	.	.	$+ 81\cdot0°$
d-mannose	.	.	.	.	.	$+ 14\cdot05°$
Pentoses—l-xylose .	.	.	.	.	.	$+ 18\cdot09°$
l-arabinose	.	.	.	.	.	$+105\cdot1°$

The action of a particular sugar on polarised light is indicated by the prefixes d- and l- ; but, owing to a convention by which this prefix is also attached to its derivatives, it comes about that sugars that are actually levo-rotatory may be designated as d-sugars (*e.g.* d-levulose), or the reverse (*e.g.* l-xylose). A mixture of equal parts of dextro-rotatory and levo-rotatory sugars is optically inactive, and this is shown by the prefix i- (inactive) or r- (racemic).

The Disaccharides are anhydrides, or ether-like derivatives, of the simple monosaccharides. They contain twelve carbon atoms,

and consist of two simple six-carbon atom residues united through an oxygen atom. They are therefore analogous to the simple glucosides. When acted on by hydrolytic agents, such as dilute acids or enzymes, they break down, with the addition of a molecule of water, into their constituent hexoses, which may be either aldoses or ketoses—

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
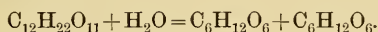
Some of the disaccharides occur in nature as such, but others result from the decomposition of still more complex carbohydrates. The more important members of the group are cane-sugar (saccharose, or sucrose), lactose (or milk-sugar), maltose (or malt-sugar), and isomaltose.

In their general properties the disaccharides closely resemble the monosaccharides. Like these they have a sweet taste, are crystallisable, are capable of passing through animal membranes, and are optically active. The specific rotation of solutions containing about 10 per cent. are as follows :—

Sucrose	.	.	.	.	.	$+ 66 \cdot 5°$
Maltose	.	.	.	.	.	$+138 \cdot 0°$
Lactose	.	.	.	.	.	$+ 52 \cdot 5°$

The disaccharides as such are not fermentable. A solution of cane-sugar or maltose will, however, undergo alcoholic fermentation when exposed to the action of yeast, but this is due to the existence in the yeast of specific ferments, known as "invertase" and "maltase" respectively, which have the power of hydrolysing the disaccharides into their constituent monosaccharides, which are then attacked and fermented. Ordinary yeast does not contain the ferment "lactase," which has the power of hydrolysing lactose, hence milk sugar is not fermented, although it may be slowly broken down into lactic acid and butyric acid by contaminating organisms.

The chemical characters of the disaccharides vary according to the way in which the constituent hexoses are bound together. In all of them the properties of the aldehyde group of one of the hexoses is masked, owing to the second being attached to it in place of an hydrogen atom in the hydroxyl group combined with the carbon atom, which exercises aldehydic functions in the open chain form. The aldehydic, or ketonic, group of the second hexose may either remain functional or disappear. In the one case the disaccharide behaves like a monosaccharide, reducing

salts of the heavy metals, forming osazones, &c., and in the other these properties are lost. The disaccharides may accordingly be divided into two groups :—

I. *Reducing Disaccharides.*

Maltose	.	.	.	.	. $=$ dextrose $+$ dextrose
Isomaltose	.	.	.	.	. $=$ dextrose $+$ dextrose
Lactose .	.	.	.	.	. $=$ dextrose $+$ galactose
Isolactose	.	.	.	.	. $=$ dextrose $+$ galactose

II. *Non-reducing Disaccharides.*

Saccharose (cane-sugar) $=$ dextrose $+$ levulose (invert sugar)

The Polysaccharides, like the disaccharides, are to be regarded as condensation products of the monosaccharides. They have a common empirical formula $(C_6H_{10}O_5)_n$, in which " n " is a variable factor that always exceeds two. In many cases the value of " n " is unknown, but it is probably always large : in starch, for example, it is 108. The polysaccharides are a very numerous class, and, although chiefly met with in the vegetable kingdom, are also found in the animal body. They may be conveniently divided into the following groups :—

1. The starch group (starch, inulin, glycogen).
2. The dextrins.
3. The cellulose group (cellulose, hemicellulose, tunicin).
4. The gum group (plant gums, mucilage, animal gums).

They are non-volatile and, with very few exceptions, are amorphous. As a class they are insoluble in alcohol, but usually dissolve in water to form solutions, which are often opalescent and exert a marked rotatory effect on polarised light. As a rule they do not diffuse through animal membrane. Their solutions are not sweet to the taste, are neutral in reaction, and yield the polysaccharide in the form of a precipitate on treatment with certain neutral salts (*e.g.* ammonium sulphate). On hydrolysis monosaccharides appear among other products. With the exception of the dextrins they do not reduce metallic oxides in alkaline solution, and none of them combine with phenylhydrazin to form osazone. They are not directly fermented by yeast, but, like the disaccharides, they may be hydrolised by the action of ferments or acids to monosaccharides, which can be fermented.

Many of the polysaccharides combine with iodine to form characteristic coloured compounds. Owing to their physical characters and feeble chemical affinities they are often difficult to obtain in a state of purity.

Acids and Acid-derivations of the Sugar Series

Certain acids, and acid derivatives, of the fatty series are related to the carbohydrates, and since some of these are natural products of the chemistry of the body, while others make their appearance in conditions where carbohydrate metabolism is interfered with, it is essential that their structure and relations should be clearly understood before the fate of the sugars under normal and pathological conditions is considered.
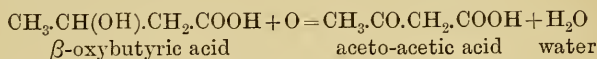
Acids having the general formula $C_nH_{2n}O_2$ are known as fatty acids, since the higher members of the series occur in natural fats (e.g. palmitic acid $C_{16}H_{32}O_2$, stearic acid $C_{18}H_{36}O_2$). The two oxygen atoms, one of the carbon, and one of the hydrogen atoms are always in the combination -COOH, which is known as a " carboxyl " radical, and it is to the presence of this that the acid properties of the compound are due. The lowest member of the series is formic acid (H.COOH) ; the next is acetic acid (CH_3.COOH) ; then comes propionic acid (CH_3.CH_2.COOH), then butyric acid (CH_3.CH_2. CH_2.COOH), and so on up the series, every member counting one more -CH_2 group than its predecessor. If, by a process of oxidation, an oxygen atom is introduced into the molecule, we have formed an oxy-acid. Thus from acetic acid (CH_3.COOH) is derived hydroxy-acetic, or glycollic, acid (CH_2.OH.COOH), which is the lowest member of the series of acids of the sugar group ; and from propionic acid we get oxy-propionic, or lactic, acid (CH_3.CH(OH).COOH). When the acid contains several CH groups, each division of the molecule is named according to its relation to the fundamental unchanged carboxyl group, the one nearest the carboxyl radical being said to be in the α-position, the next on the left in the β-position, and the next to it in the γ-position. From any one acid we can therefore have produced a series of oxy-acids differing in the position of the OH group, and known as α-, β-, γ-oxy-acids respectively. Thus from butyric acid (CH_3.CH_2.CH_2. COOH) there can be theoretically derived three such acids :—

$$\alpha - CH_3.CH_2.CH(OH)COOH$$
$$\beta - CH_3.CH(OH).CH_2.COOH$$
$$\gamma - CH_2.(OH).CH_2.CH_2.COOH$$
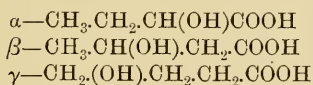
In the body oxidation appears to take place most readily in the β-position, and hence in the above series, for instance, it is β-hydroxybutyric acid that is of most physiological importance.

In the laboratory the whole series of oxy-acids may be ob-

tained by suitable means, and, moreover, more than one group may undergo the change—*e.g.* glyceric acid $(CH_2.(OH)CH(OH) COOH)$, gluconic, mannonic, or galactonic acid $(CH_2.CH(CH.OH)_4. COOH)$. If β-oxybutyric acid undergoes further oxidation, and this takes place in the β-position, as it is supposed to do in the body, aceto-acetic, or di-acetic, acid and water result :—

$$CH_3.CH(OH).CH_2.COOH + O = CH_3.CO.CH_2.COOH + H_2O$$
$$\beta\text{-oxybutyric acid} \qquad \text{aceto-acetic acid} \quad \text{water}$$

By further oxidation, and the splitting off of a molecule of carbon dioxide, it is possible to derive acetone from aceto-acetic acid—

$$CH_3.CO.CH_2.COOH = CH_3.CO.CH_3 + \quad CO_2$$
$$\text{aceto-acetic acid} \qquad \text{acetone} \quad \text{carbon dioxide}$$
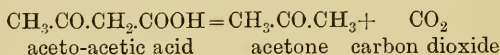
A further series of acids can be derived from the simple fatty acids by the substitution of a carboxyl group for one of the hydrogen atoms of the terminal -CH group ; thus carboxyl-acetic, or malonic, acid $(COOH.CH_2.COOH)$ may be regarded as derived from acetic acid $(CH_3.COOH)$ by such a substitution. Another important member of this series is oxalic acid $(COOH.COOH)$. These acids, since they contain two carboxyl groups, are dibasic, while those previously considered, which contained only one carboxyl group, are monobasic. Oxidation of the dibasic, as of the monobasic acids, gives rise to a series of oxy-acids ; thus tartronic acid $[COOH(CH.OH)COOH]$ is the oxy-acid of malonic acid $(COOH. CH_2.COOH)$. Other important di-basic oxy-acids are tartaric $[COOH.(CH.OH)_2.COOH]$, and saccharic and mucic acids $[COOH (CH.OH)_4.COOH]$.

An important oxidation product of the sugar series, intermediate between the mono- and di-basic acids, and possessing both acid and aldehydic properties, is glucuronic, or glycuronic acid $[CHO(CH.OH)_4.COOH]$.

(A more detailed account of the properties of the carbohydrates, and the acids, and acid derivatives of the sugar series, is given in the Appendix.)

The Digestion and Assimilation of Carbohydrates

Only a small proportion of the carbohydrates of the food are in a form fitted for immediate absorption, the greater part consisting of starches, sugars, &c., which must undergo cleavage and hydrolytic changes before they can be taken up by the walls of the intestinal tract. These changes are brought about by the

action of ferments found in the saliva, pancreatic secretion, and intestinal juices.

Two distinct classes of ferments can be recognised—(1) those known as " amylolytic " or " diastatic " ferments, which act upon starches, producing sugars and dextrins ; and (2) those which act upon various saccharoses, giving rise to glucose, called " inverting " ferments. The two chief amylolytic ferments are the ptyalin of the saliva and the amylopsin of the pancreatic juice, while the inverting ferment is found in the mucous membrane of the small intestine and in the succus entericus.

The starches contained in the food have usually been broken up and partly converted into dextrins by cooking before they are consumed. Their digestion is commenced in the mouth, soluble starch, certain forms of dextrin, maltose, and traces of dextrose resulting from the action of the ptyalin of the saliva. But digestion is not carried very far here, especially when the food is not well masticated. In man salivary digestion plays quite a secondary rôle. When the starchy foods reach the stomach the diastatic fermentation initiated in the mouth is quickly, although not immediately, stopped, the exact stage depending upon the rapidity with which hydrochloric acid in the free state appears in the gastric contents. The hydrochloric acid of the gastric juice may now bring about a certain amount of hydrolysis of the cane-sugar and maltose arising during salivary digestion, or already present in the food, but the inversion of saccharoses occurs mainly in the intestine. Absorption of sugars takes place to a slight extent through the stomach wall, especially when there is a concentrated solution, but it is never very marked. The presence of alcohol increases absorption, even from dilute solution, and may help to account for the glycosuria that occasionally follows the ingestion of alcoholic beverages, and particularly champagne and beer.

Carbohydrate digestion is essentially effected in the small intestine. Through the agency of the pancreatic ferment, amylopsin, the insoluble starch is converted into soluble starch, or amylodextrin, and is then successively decomposed, by gradual hydrolysis, into erythrodextrin, achroödextrin, isomaltose, and maltose.

Glycogen is similarly decomposed and, like starch, gives rise to isomaltose and maltose. Cellulose is not affected by any of the digestive ferments, but under the influence of the intestinal bacteria it undergoes a certain amount of fermentative change, particularly in herbivorous animals, so that only a fraction of the ingested cellulose appears as such in the fæces. Maltose or dex-

trose have not, however, been found as products of the fermentation
of cellulose in the intestinal tract. The intestinal bacteria also
appear to have the power of transforming a certain amount of
starch into maltose and other products ; for when the pancreatic
secretion is prevented from entering the intestine, either by extir-
pating the organ or ligaturing the duct, from 47 to 71 per cent.
of the ingested starch of the food appears to be utilised. In
severe cases of pancreatic disease in man, where the digestive
functions of the pancreas are seriously interfered with, it is found
that there is not the marked failure of starch digestion that might be
expected, and that analysis of the fæces shows to have occurred with
the fats and proteids. This is probably due to the action of intestinal
bacteria ; but it has also been suggested that the epithelial cells of
the small intestine are capable of inverting dextrin to maltose, and
so replacing to a certain extent the functions of the pancreas.

Maltose is the chief sugar formed by the action of amylopsin
on starch, but before absorption takes place this and other poly-
saccharides present in the intestinal contents are converted into
monosaccharides. The change is effected chiefly by the inverting
ferments, maltase and lactase. The succus entericus possesses only
feebly diastatic powers, but by means of these ferments, which are
contained in it, it rapidly converts maltose into grape-sugar and
levulose. These are absorbed by the epithelial lining of the in-
testinal mucosa, and, passing thence by way of the mesenteric
veins, reach the portal system. Probably less than one per cent.
normally goes through the lymphatics and the thoracic duct
directly into the venous system. It is probable that a small pro-
portion of the dextrin formed in starch hydrolysis, and possibly
also some of the saccharoses, are absorbed by the intestinal epi-
thelium as such, and are converted by the cells before being passed
into the blood stream.

The rapidity with which carbohydrates are digested and ab-
sorbed varies considerably. Albertini found that when 100 grams
of dextrose are given to dogs, 60 grams are absorbed in the first
hour ; while of similar doses of maltose and cane-sugar from 70
to 80 grams, and of lactose from 20 to 40 grams, disappeared
within the same period. The fact that no soluble carbohydrates
can be found in the fæces does not prove that they have all been
absorbed and utilised. Even in health there is always some waste
from fermentation by bacteria in the intestine, acetic acid, lactic
acid, butyric acid, succinic acid, formic acid, alcohol, carbon
dioxide, methane, hydrogen, and other bodies being formed.
In conditions of disease, where the intestine flora is abnormal,

such changes may be much exaggerated and give rise to disturbances of intestinal digestion and diarrhœa, which are favoured by the ingestion of large amounts of sugar. In such cases the loss of caloric potential may be very considerable and lead to marked inanition. It is not improbable that the liking which many diabetics exhibit for sweet substances may, through the long-continued changes set up in the intestine and pancreas by excessive intestinal fermentation, have been the cause of the imperfect sugar metabolism which they subsequently develop. Starches do not appear to have the same effect as sugar, probably because inversion and absorption run parallel, so that the intestinal bacteria have little chance of setting up fermentative changes and giving rise to the formation of irritating by-products such as is afforded by an abnormally large amount of sugar.

The digestive processes undergone by the carbohydrates of the food are definitely known, and it is also certain that they are an important source of energy, but exactly how they are distributed and utilised is not quite so clear. When sugar, whatever its source, is absorbed into the blood, it is fixed by the cells of the body in the form of glycogen. This glycogen bears a similar relation to the living cell that the coal in its tender bears to a locomotive. It forms a store on which the tissues can draw in the course of their metabolism, converting the potential energy of carbohydrates into work, and ultimately breaking it down into carbon dioxide and water. It was at one time thought that the carbohydrates of the food were the only source of energy for the body, and that the proteids were used only to repair tissue waste. Such, however, is not the case, and the difficulties of the problem of carbohydrate metabolism have been increased by the discovery that most proteins contain a carbohydrate radicle, and the possibility that this may be split off and utilised by the organism. The results of experimental investigation suggest that this does occur, and that glycogen may be formed from such carbohydrate groups. But proteids, such as casein, which contain no preformed carbohydrate complex, increase the sugar output in the diabetic organism, and it is therefore probable that sugar may in addition be derived from other decomposition products of the protein molecule. It also appears certain that glycogen may be formed by an animal from the proteins of its own tissues. Which are the degradation products of the protein molecule that can be converted into carbohydrate are not known for certain, but the amino-acids, containing six or three carbon atoms, suggest themselves as the most likely source.

The neutral fats, consisting as they do of glycerine combined with fatty acids, are another possible source of sugar in the organism. The transformation of glycerine into sugar is not a difficult chemical operation, and experiments with phloridzinised animals, and dogs after the removal of the pancreas, suggest that such a transformation may occur within the body. The glycerine is probably first converted into glycerose, and this in its turn is converted into levulose. The fatty acid portion of the neutral fats cannot probably be converted into sugar, although some authors (*e.g.* v. Noorden) maintain that even this transformation can be effected. In the present state of our knowledge it may be concluded that fats may be one source of sugar in the organs, but that there is an absence of conclusive proof to this effect as yet.

It is evident, therefore, that the subject of carbohydrate metabolism is much more complicated than at first sight it might appear to be, and that in considering the question we have not only to take into account the carbohydrates of the food, but also the food and tissue proteins, and probably also the fats.

The most generally accepted view dates from the discovery by Claude Bernard, in 1857, that the liver contains very little free sugar but a considerable amount of glycogen. It is based upon the theory that the liver glycogen represents the carbohydrate absorbed in excess of the immediate requirements of the body, and that this is converted into sugar by the action of a ferment, and is transported to the tissues as they require it by the systemic blood stream. According to this, which may be termed the classical view, the sugar taken up by the blood from the intestine is conveyed by the portal vein to the liver, where it is converted into glycogen and stored for the future needs of the body. In support of this is adduced the experimental evidence that during absorption the blood in the portal vein contains a much higher percentage of sugar (0·2–0·4 %) than the systemic blood (0·05-0·2 %), but during fasting the percentage is the same. The amount of glycogen in the liver depends largely on the intake of food, but never exceeds about 150 grams (about 5 ounces), and as this only disappears after several weeks' starvation, it cannot account for the whole of the sugar which is absorbed in a short space of time when a meal rich in starch and sugar is taken. It is therefore assumed that the excess passes through the liver and is laid down in the muscles and other tissues, also in the form of glycogen, thus accounting for another 150 grams. Even if the whole of the glycogen in the liver, muscles, and other tissues of the body, and the sugar in the circulating blood, which is about

10 grams ($\frac{1}{2}$ ounce) or less, are allowed for, they do not still represent the whole of the carbohydrate that may be absorbed. It is consequently supposed that the balance enters into the constitution of the proteins, nucleo-proteins, and albuminoids, from which a carbohydrate material has been obtained on treatment with acids ; it is probable also that a certain proportion may be turned into fat. The glycogen in the liver, and to a less extent in the muscles, is believed to form a readily available store from which the wants of the body can be quickly supplied, the glycogen being converted into dextrose by the action of special ferments as the need arises. The sugar thus formed in the liver is conveyed to the tissues, which seize upon it and utilise it in their metabolism, splitting it up and oxidising it. According to this theory provision is made in the body for a certain percentage of sugar to be constantly present in the blood. If from any cause the amount circulating exceeds more than from 0·1 to 0·2 per cent., the excess is excreted by the kidneys. This is normally prevented by the fact already referred to, that the liver and muscles at once store up any excess above the normal, resulting from too rapid absorption, as glycogen. If, on the other hand, the percentage in the blood sinks below the normal, owing to the consumption being increased from work or heat production on the part of the tissues, the liver, and later the muscles, at once give back a portion of their glycogen to the blood in the form of sugar. If the stored glycogen is insufficient for this purpose, fat and albuminoids are made use of, the percentage in the blood remaining constant, even after long-continued starvation.

The theory that the carbohydrates of the food are destined to pass through the circulation to the tissues in the form of sugar has been strenuously opposed by Pavy. He considers that the assumption as to the impermeability of the kidneys to sugar involved in this theory is a fiction, and that in reality the urine stands in very sensitive relationship to the blood with respect to sugar. According to his view, all the food which has been broken down in the intestine, and placed in a fit state for absorption, is at once dealt with at the seat of absorption, being rebuilt, before reaching the circulation, into molecules of sufficient size to prevent their flowing off with the urine in its passage through the kidneys. The building-up process, he believes, is effected by the lymphocytes, thus accounting for the lymphocytosis which accompanies digestion. The lymphocytes, carrying the elaborated food material, pass from the villi into the absorbent vessels, and thence through the thoracic duct into the vascular system. There they break down

and are transformed into the protein constituents of the chyle
and blood plasma, thus bringing the elaborated food into direct
relation with the tissues. The carbohydrates, fat, and nitrogen con-
taining material enters into the protoplasmic complex of the cells
and, interacting with the oxygen, also brought by the blood, give
rise to energy and the phenomena of life. Any oxidisable material
taken on in excess of the consumption is cleaved off and stored
for future use in the shape of glycogen and fat. Sugar which is
not disposed of at the seat of absorption in the manner described,
and particularly when a large amount of carbohydrate food is
ingested, is supposed to pass to the liver, and there be checked
from further progress by being taken into the liver cells and con-
verted into glycogen, and possibly also into fat. The liver thus
forms a second line of defence against the passage of absorbed
sugar into the systemic circulation, and prevents the onset of the
glycosuria that would occur if it flowed on instead of being re-
tained. The glycogen in the liver and muscles is, according to
this idea, to be regarded simply as a reserve of carbohydrate
material ready to be drawn upon and utilised as it becomes wanted,
its special accumulation in the liver being accounted for by the
position which that organ occupies in relation to the food supply.
In the muscles the amount stored depends chiefly upon the extent
to which they are used, diminishing with exercise and accumulating
at rest. As in the case of starch in the vegetable kingdom, the
glycogen is probably broken down into sugar before being absorbed
into the protoplasm of the tissue, and it can be inferred that this
change is brought about by the action of an enzyme. The glycogen
in the liver when required is similarly broken down by enzyme
action into sugar, but this, instead of passing directly into the
circulation, is assumed to be loosely linked on as a side-chain to a
protein nucleus, and to be conveyed in this locked-up condition to
the tissues, where the carbohydrate radicle is taken off and utilised
as required, the protein molecule thus set free being available for
the attachment of a fresh sugar side-chain. The essential point
in this theory is that the carbohydrate is transported from the
seat of accumulation in the liver to the seat of utilisation in the
tissues as part of a large molecule which can pass through the blood
without running off with the urine. Pavy's views have not been
generally accepted, in spite of the brilliancy and perseverance
with which he defended them, but they have undoubtedly had a
considerable effect in the way of modifying the theories based
originally on Bernard's experiments.

Whether the carbohydrates of the food inevitably go through

the glycogen stage or not, there can be no doubt that they ulti-
mately reach the tissues and are there broken down, eventually
forming carbon dioxide and water. In spite of the large amount
of research which has been devoted to the elucidation of the pro-
blem of the metabolism of sugar in the animal organism, the exact
details of the intermediate steps are as yet not understood. It
is probable that the decomposition does not occur, at any rate in
its entirety, as a direct oxidation, but that an intermediate series
of oxidation and fission products are formed.

Some interesting observations, made from a chemical stand-
point by Adolf Jolles of Vienna, seem calculated to throw con-
siderable light on this subject. He has investigated the action of
various oxidising agents upon a number of different sugars, in-
cluding arabinose, rhamnose, dextrose, levulose, invert sugar,
mannose, galactose, cane-sugar, maltose, and lactose, in weak
alkaline solutions at 37° C. In general the strength of the solutions
was 1 per cent. of sugar made N/100 alkaline with sodium hydrate.
In every case, with the exception of cane-sugar, a diminution in
the rotating power of the solution occurred, together with the
formation of acids. He found that neutralisation of the alkali
produced marked slowing in the formation of the acids, while
with glucose in N/100 acid solution acid formation does not occur
and the sugar remained unaltered. By the addition to the solu-
tions of hydrogen peroxide the oxidation processes were accelerated,
occurring more quickly than when the oxygen in the air was used
as the oxidising agent. Levulose was found to produce more acid
than dextrose, and therefore to be more easily oxidisable. The
oxidation products obtained comprised ethyl alcohol, acet-aldehyd,
acetone, formic, acetic, butyric, lactic, glycolic, oxalic, succinic,
aceto-acetic, and glucuronic acids. With most sugars the chief
product was formic acid, a very small quantity of acet-aldehyd, and
an acid which gave Tollen's naphtho-resorcin reaction for glucuronic
acid also being formed. Lactic acid was only obtained in alkaline
solutions of dextrose without the addition of hydrogen peroxide.
The use of oxide of silver as an oxidising reagent gave similar
results to those obtained with hydrogen peroxide. Ammonia and
sodium carbonate did not influence the decomposition of sugar
as strongly as sodium hydrate.

Jolles is of opinion that the conditions of his experiment ap-
proximated to those in the body. The blood with its definite
alkalinity permeates the tissues, and the peroxidases, catalases,
and other oxidising ferments can be regarded as exerting a similar
action to the hydrogen peroxide in his chemical experiments. He

B

suggests that the sugar in the tissues is oxidised to acids of low molecular weight, such as formic acid, which are further oxidised in the blood to carbon dioxide and water. He has also shown how his observations can be adapted to explain glycolysis in muscle, the formation of sarcolactic acid, of glucuronic acid, and even some of the features of diabetes and pentosuria.

A series of experiments carried out by Nef have suggested that glycerin aldehyde is an important intermediate product of the breakdown of the hexoses. Observations conducted by Woodyatt tend to confirm this, and show that in the course of the utilisation of sugar in the body a cleavage of glucose into two molecules of triose is an important event. According to Nef, lactic acid, glycerinic acid, and other oxidation products of glycerin aldehyde are formed by intramolecular rearrangement in this body when there is an insufficient supply of oxygen.

As the result of a series of researches carried out by Stoklasa and others, it has been assumed that the tissues contained a glyco-lytic enzyme capable of causing true alcoholic fermentation of sugars, and that it is by the action of this ferment that the degra-dation of sugars is brought about in the body. In support of this view there has been cited the well-known phenomenon of the formation of lactic acid in the tissues after death, which has been interpreted as an intermediate stage in the process of alcoholic fermentation. Harden and Maclean have shown, however, that this theory is probably based upon altogether erroneous observa-tions. Experiments of this kind involve the examination and manipulation of various animal organs and tissues, and it is only with great difficulty that they can be kept free from contamination with bacteria. In most cases hitherto no attempt has been made to do so. Harden and Maclean point out the difficulty of per-forming such experiments under absolutely sterile conditions, but with careful precautions they succeeded on a few occasions. In these cases no trace of alcoholic fermentation could be detected. The presence of an efficient antiseptic leads to a similar negative result. The fermentation occurring under natural conditions must, therefore, be due to the action of bacteria, a large number of which are known to cause rapid fermentation of various sugars. The unavoidable conclusion, therefore, is that there is no satisfactory evidence that alcoholic fermentation occurs in animal tissues after removal from the body, apart from the presence of sugar-fermenting bacteria.

Carbohydrates in Normal Blood.—Normal blood always con-

tains traces of sugar, which may be temporarily increased by a diet rich in carbohydrates, and be diminished by muscular exercise and hunger.

The sugar content of the systemic circulation averages about 0·8 grams per 1000 when it is estimated by the ordinary reduction methods and is calculated as dextrose. Limbeck found in the blood of two healthy subjects, five hours after eating, 0·075 per cent. and 0·089 per cent. With the polarimeter, however, a much lower reading is obtained, so that the sugar of the blood must either be a variety differing from dextrose, or be composed of a mixture of sugars with opposite optical characters.

Many physiologists consider that part at least of the sugar in the blood exists in loose combination with some other substance. Some maintain that this is lecithin, forming the so-called jecorin, first found by Dreschel in the liver, while others believe that the albuminates are the sugar-carriers. Most are agreed that part exists in a free state, but the work of Rona and Michaelis tends to prove that all the sugar in the blood is in a simple state of solution, some in the corpuscular elements, the remainder in the plasma. They have shown that when diluted blood is shaken with certain colloids, such as ferric hydroxide or kaolin, the proteins form a colloidal combination and are absorbed. They can then be quantitatively precipitated by the addition of a trace of electrolyte, but that no trace of sugar is removed from the solution by this treatment. If the sugar were in any way united with the proteins it would be carried down with them, and as the reagents employed cannot have any disruptive effect, it is not possible that the sugar can exist in combination with the proteins. Another piece of evidence in support of the free state of dextrose in the blood is furnished by the observation that, whereas charcoal absorbs both sugar and protein when shaken with a solution containing these two substances, yet it absorbs the protein, but not the dextrose, when acetone is present; the acetone being more absorbable than the dextrose, prevents the latter being taken up by the charcoal. Further evidence is also furnished by the results of dialysis experiments.

Levulose.—Lépine and Boulud have obtained from the blood in certain cases a reducing sugar having the characters of levulose, and explain its presence on the assumption that it has been derived from dextrose in the alkaline medium furnished by the blood.

Maltose has been demonstrated in the blood of healthy rabbits and dogs, and is supposed to be derived from the intestinal

contents, or to depend upon imperfect hydrolysis of glycogen in the liver.

Traces of *pentose*, and in certain instances of a sugar resembling *saccharose*, have been found in the blood. The former appears to be a constant constituent, and the latter is supposed to be derived, either from the intestinal contents, or be produced in the animal economy by a combination of dextrose with levulose.

Glucuronic acid has been described as present in the blood of both man and cattle by P. Mayer, and this observation has been extended by Lépine and Boulud to the dog. Since the conjugate glucuronates are levo-rotatory, their presence would help to explain the difference between the readings obtained with an extract of the blood by the polariscope and on reduction. The small quantity in normal blood is, however, against this being the sole explanation.

Animal Gum.—Freund has obtained from blood a carbo-hydrate-like substance resembling the animal gum of Landwehr. Ox blood was found to contain about 0·02 per cent.

Glycogen is said to be present in traces in the blood, but it is not improbable that the glycogen found free in the plasma is derived from the leucocytes, which are known to contain it.

BIBLIOGRAPHY

Allen's *Commercial Organic Analysis,* vol. i., 1909.
Armstrong, *The Simple Carbohydrates,* 1910.
Fenton, *Journ. Chem. Soc.,* 1907.
Fischer, *Untersuch. u. Kolenhyd. u. Fermente,* 1909.
Freund, *Centralb. f. Physiol.,* 1892.
Gründ, *Zeit. f. phys. Chem.,* xxxv., p. 111.
Harden and Maclean, *Journ. of Physiol.,* 1911.
Jolles, *Weiner med. Woch.,* 1911.
Lépine, *Le diabète sucré,* 1909.
Lépine and Boulud, *C.R. de l'Acad. d. Sci.,* 1901–2.
Limbeck, *Prag. med. Woch.,* 1893.
MacLeod, *Recent Advances in Physiology,* 1906.
Mayer, *Zeit. f. physiol. Chem.,* 1901.
Nef, *Ann. d. Chem., Liebig's,* ccclvii.
Pavy, *Lancet,* 1908.
Röhmann, *Biochemie,* 1908.
Rona and Michaelis, *Biochem. Zeit.,* xiv.
Schryver, *Proc. Roy. Soc.,* 1910.
Tollens, *Kurze Handbuch d. Kolenhyd,* 1898.
Woodyatt, *Journ. Amer. Med. Ass.,* 1910.

CHAPTER II

NORMAL URINE

THE question as to whether the urine of healthy individuals contains sugar was for many years a subject of keen controversy. In 1848 Lespiau stated that normal urines have reducing powers. Ten years later Brücke confirmed this observation, and declared that normal urines contain sugar. His statements were supported by Bence Jones, Tuchen, Abeles, Meissner and Babo, Udranszky, Wedenski, Molisch, Quinquand, Bruel, Luther, Roos, Moritz, Binet, Allen, Baisch, and Pavy, who maintained that all urines contain small quantities of reducing carbohydrates. Some observers, including Seegen, Friedlander, Malay, Leuken, Külz, G. and S. G. Johnson, and others, while they allowed that normal urines possess slight reducing powers, came to the conclusion that this can be entirely explained by the presence of other substances than sugar, and particularly creatinin and uric acid. There can be no doubt that these bodies do partly account for the slight reduction caused by many urines when they are boiled with alkaline solutions of copper, &c., but, although a number of the recorded observations bearing on the question are open to serious criticism, and, as Johnson pointed out, it involves a definition of what is a "normal" urine, the balance of available evidence is in favour of the view that the urines of average healthy individuals probably contain minute quantities of glucose, and traces of other reducing carbohydrates. The constant presence of the former has not, however, been absolutely proved, and it is probable that diet, exercise, and mode of life have some bearing on the question, and also on the excretion of glucuronic acid, the presence of which also contributes to the reduction. Worms examined the urines of 507 persons of the labouring class, and found that in every instance they were free from sugar; but out of 100 samples from persons engaged in sedentary occupations, involving mental activity, he found sugar in ten. My own observations have given somewhat

similar results, but in my experience the reduction given by the urines of persons engaged in sedentary work appears to be chiefly dependent upon the presence of glucuronic acid. Haas, in 1876, pointed out that normal urines are faintly levo-rotatory, and in 1885 Fluciger explained this by the presence of glucuronic acid, for he found that on heating the urine with dilute acid its reducing power is much increased and its optical activities are altered. His opinion has since been confirmed by Mayer and Neuberg, by Porcher and Nicolas, and by others, who have shown that glucuronic acid is a very constant constituent of normal urine. According to Mayer and Neuberg, it is usually present in quantities of about 0·004 per cent., mostly in combination with phenol, and to a less extent with indol and skatol. Moritz states that the uric acid and creatinin of the urine account for about 50 per cent. of its reducing power under normal conditions ; but more recently Levesson has given a lower figure, 25 to 33 per cent. of the total.

The total amount of reducing carbohydrate in normal urine has been variously estimated by different observers, but, as we have seen, some of these variations may be partly explained, in all probability, by the diet and environment of the persons whose urine was investigated, while the different methods of estimation employed offer another partial explanation. According to Salkowski, the reducing substance of normal urine varies from 0·254 to 0·596 per cent. Rosen and Alfthan obtained from 1·5 to 3·0 grams of precipitate from the twenty-four hours' urine of a healthy man by the benzoyl chloride process, while Baisch states that normal urines contain about 0·12 to 0·32 grams of reducing carbohydrate, of which 0·08 to 0·18 grams is grape-sugar. Baisch and Lemaire have also isolated a sugar which they considered to be isomaltose, besides a dextrin-like substance having the characters of Landwehr's animal gum and a nitrogen-containing body yielding furfurol, probably derived from mucin or chondroitin sulphuric acid, from normal urine. Lohenstein places the amount of sugar as low as 0·001 per cent., while Pavy regards 0·05 per cent. as the average amount. Kellas and Wethered state that an average of 0·08 per cent. of substances reacting like grape-sugar may be normally present in the urine. The most recent observations made by Schondorff place the quantity of sugar at about 0·01 per cent.

Clinically the presence of grape-sugar in normal urines is of minor importance, for in any case the quantity is so exceedingly small that it is unrecognisable by the ordinary methods of testing, and such amounts are associated with no clinical symptoms. It is important, however, to remember that urines from apparently

healthy individuals contain substances which have reducing powers, and that under certain circumstances these may be sufficient to give puzzling results with some methods of examination.

Abnormal Urines

In abnormal conditions the reducing power of the urine may be increased so that a more or less marked reaction is obtained with the ordinary clinical tests. By far the most common and important cause is the presence of an appreciable quantity of dextrose, but a reaction may also be due to the presence of levulose, lactose, galactose, maltose, isomaltose, pentoses, homogentisic acid, or compound glucuronates, and a doubtful result is sometimes dependent upon an increased excretion of uric acid and creatinin. The presence of some of these substances indicates an undoubted perversion of the metabolism of the body, which may, or may not, be of a permanent and serious character. Others are of doubtful significance. A few are of no known pathological importance. It is obvious that these groups must be clearly differentiated, and the more important members be definitely recognised, if an analysis of the urine is to be of any use in treatment and prognosis. In the succeeding pages of this chapter the means by which these objects may be attained will be considered, and reference will also be made to other carbohydrates and substances of a similar composition, which are occasionally met with in the urine.

Collection of the Urine.—When selecting a single specimen of urine for examination for sugar, it is best to take one that has been passed during the day, preferably in the evening, for if only a small amount is present the morning urine will probably contain less than the evening, and may even give no reaction at all. It may also happen that a specimen taken out of the collected urine for twenty-four hours will give a doubtful or negative result, whereas one that has been passed three or four hours after a meal, and particularly a meal rich in carbohydrates, will give a decided reaction for sugar. Conversely, a strictly protein diet may cause sugar which has previously been present to disappear. For diagnostic purposes it is therefore advisable that an examination of a twenty-four hours' sample should first be made, and, if this is negative, another specimen taken three or four hours after a meal containing an average amount of carbohydrate should be investigated. All specimens must be examined in as fresh a state as

possible, since traces of sugar may be destroyed, and escape detection, if the urine has been allowed to ferment and decompose.

The Physical Characters of the Urine often afford some indication as to the presence of sugar. Urines containing much glucose usually present a pale, greenish-yellow colour, combined with a high specific gravity, 1·025 or over. It is not uncommon, however, for urines with a normal or even a low specific gravity to contain sugar, and v. Jaksch has reported examples where the specific gravity has been as low as 1·003. In pentosuria, lactosuria, levulosuria, and similar conditions the specific gravity does not, as a rule, show any marked variation from the normal. The amount of urine excreted in most cases of persistent dextrosuria is excessive, 3 or 4 litres (5 to 7 pints) a day in many instances. Its reaction is generally distinctly acid, and on being shaken it readily forms a persistent froth. Saccharine urines ferment spontaneously, especially in warm weather, forming bubbles of carbonic acid gas, and showing a sediment of yeast microscopically, except when the unfermentable sugars lactose, pentoses, &c., are alone present.

The Chemical Reactions of Saccharine Urines.—The oldest and simplest test for sugar in the urine is afforded by its sweet taste. Celsus and Galen in describing diabetes make constant reference to the " sweet and honey urine." In China and the East, sugar is detected by allowing the urine to evaporate on the ground in the sun, and then watching for the concourse of ants and other insects that are attracted by the sweet residue. A somewhat rough-and-ready test, but one which is much more delicate than might be supposed, and that can be carried out at the bedside with no more apparatus than can be obtained in any household, is afforded by evaporating a few drops of the suspected urine to dryness in a spoon over the flame of a candle or lamp. The residue is gently heated, and as the temperature rises it will be seen to form a pure yellowish-brown viscid mass, which is sticky to the touch, and gives forth an odour of caramel before it is reduced to ash, at about 200° C., if much sugar is present. Urines free from sugar treated in this way show a dirty grey-brown residue, and give no odour of caramel on being further heated.

The laboratory tests for sugar may be conveniently divided into :—

    1. General tests, with which a reaction is given by all sugars.
    2. Classifying tests, which separate the sugars into groups characterised by the possession of one or more common characters.

3. Special or confirmatory tests, which serve to more or less completely differentiate particular sugars. Many of these can only be satisfactorily applied to the separated and purified sugar, however.

1. *General Tests*

In testing urines for sugar the minute traces that may be normally met with are disregarded, and sugar is only considered to be present when a characteristic reaction is obtained by methods which have been proved by clinical experience to show a pathological amount. The most usually employed tests are based upon the reducing powers of the sugars, but since other reducing substances are also met with in the urine, it is advisable, and in all doubtful cases necessary, to confirm a positive reaction by other methods before it is concluded that any reduction that has taken place is due to sugar.

1. **Moore-Heller Test.**—This is one of the earliest described tests for sugar in the urine, but it is now rarely made use of, as it only gives a characteristic reaction with a relatively high percentage.

A few cubic centimetres of sodium, or potassium, hydroxide are added to about three or four times their volume of the urine, in a test-tube, and boiled for 2–3 minutes. If a considerable amount of sugar is present the fluid begins to turn brown at about 60° C., and gradually darkens as the heating is continued. The reaction is only characteristic of sugar if the colour is a dark yellow to a dark brown, or with diluted urines an intense yellow. It is a wise precaution to compare the result with that given by a normal urine under similar circumstances. If the mixture is allowed to cool and is then cautiously acidulated with sulphuric acid an odour of burnt sugar should be produced.

With pure sugar solutions the test is very sensitive, but only urines containing at least 0·5 to 1 per cent. of sugar give a characteristic brown colouration. Sugar-free urines may give a dark yellow coloration when boiled with a caustic alkali, especially if they are high-coloured to start with. All urines containing mucus darken somewhat. Any albumen that is present must be removed by acidifying, boiling, and filtering before applying the test. A flocculent precipitate of earthy phosphates is generally produced when the alkali is added to the urine, and this collects into large flocculi when heat is applied. It is, however, quite a normal phenomenon.

2. **Trommer's Test.**—If a few drops of a dilute solution of

copper sulphate are added to a solution of caustic potash or soda,
a blue precipitate of hydrated cupric acid is formed—

$$CuSo_4 + 2KOH = K_2SO_4 + Cu(OH)_2.$$
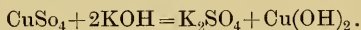
On heating the liquid the precipitate blackens, owing to the
formation of cupric oxide (CuO). If, however, glycerine, tartrates,
and various other substances are present in the solution, the cupric
hydrate is not precipitated when the copper and alkaline solutions
are mixed, but forms a deep blue solution, which on being heated
does not blacken. Dextrose acts like glycerine and tartrates in
keeping the cupric hydrate in solution, forming with it a compound
with the formula $C_6H_{12}O_6.5Cu(OH)_2$, but when the temperature of
the solution is raised to near the boiling-point reduction occurs,
the blue colour of the solution being discharged, and a yellow pre-
cipitate of cuprous hydrate $(Cu_2(OH)_2)$, or a red precipitate of
cuprous oxide (Cu_2O) appearing.

With urine the test is carried out as follows :—

To a test-tube about half filled with the suspected urine is added from
a quarter to a third of its volume of a 10 per cent. solution of sodium, or
potassium, hydrate. A 5 to 10 per cent. solution of copper sulphate is then
added drop by drop, shaking after each addition, until a faint trace of copper
hydroxide remains undissolved. If the urine is found to take up much
copper before a permanent precipitate appears, and it assumes a deep blue
colour, the presence of sugar is probable, the amount of copper required
being a rough indication of the quantity of sugar. On gently heating, but
not actually boiling, the upper part of the mixture, a yellow or greenish tur-
bidity will appear in the heated portion and spread downward through the
blue fluid, if the urine contains sugar in an appreciable amount. Eventually,
as the heating of the mixture is continued, the blue colour is more or less
completely discharged, and a yellow or red precipitate settles to the bottom
of the test-tube. If the urine contains a high percentage of sugar, metallic
copper may separate out on the walls of the tube as a brownish-red coating.

A typical reaction is only obtained with urines that contain a
distinctly pathological amount of sugar, but no other substances
than the reducing sugars give a quite characteristic reaction.
When only traces are present (under 0·5 per cent.) the fluid may
turn yellow, but no precipitation of copper oxide occurs. The
formation of an intense brilliant yellow colour, while suggestive of
the presence of a small amount of sugar, is not conclusive evidence,
for other substances may bring about this less typical reduction.

In carrying out Trommer's test it is important to bear in mind
the following points :—

1. That normal urines contain substances, such as uric acid,

creatinin, and salts of ammonia, which are able to dissolve cupric hydrate, hence, as a rule, from three to five drops of copper sulphate can be added to each 10 c.c. of urine before precipitation occurs. The resulting fluid is, however, greenish-blue rather than a distinct blue.

2. From the presence of uric acid, creatinin, glucuronic acid compounds, traces of carbohydrates, and pyrocatechin, &c., normal urines have some reducing power, the total reduction that takes place being usually equal to about 0·25 to 0·5 per cent. of glucose. On heating a normal urine with an alkaline solution of copper hydrate to boiling, therefore, the colour of the solution may change to a deep yellow by transmitted, and a reddish yellow by reflected, light, owing to a partial reduction of the copper hydroxide, but no actual precipitation occurs, the fluid remaining perfectly clear. The sediment of phosphates at the bottom of the test-tube produced by the addition of the alkali to the urine may, however, be coloured reddish-brown by traces of entangled cuprous oxide and a grey-green turbidity, due to the formation of an amorphous copper compound of xanthin bases, and uric acid may also be given by concentrated (febrile) urines. The copper precipitate formed by sugar is granular and not amorphous. Since the reducing power of a normal urine is greater than its power of holding copper hydroxide in solution, faintly ammoniacal urines, which dissolve more copper, and so allow fuller play to its reducing action, may give a precipitate, even when no abnormal amount of sugar is present, although more often the black cupric oxide is formed. A urine containing both ammonium carbonate (alkaline fermentation) and sugar may dissolve, and reduce, a good deal of copper hydrate, but yield no precipitate of suboxide, because the latter is held in solution by the ammonia.

3. That normal urines contain substances such as uric acid, creatinin, salts of ammonia, &c., which can hold a certain amount of reduced copper suboxide in solution, and, since this power is more marked than are the reducing abilities of such urines, they only give a colour change, and yield no precipitate, on being boiled with an alkaline solution of copper. When a normal urine is treated with dextrose until a solution of about 0·5 per cent. strength results, and this is tested by Trommer's method, it is found that a considerable amount of copper hydroxide is dissolved, and a strongly marked yellow colour is produced on heating, but no precipitation of copper suboxide occurs while the heat is being applied, or immediately after. This is due to the suboxide, formed both by the reducing substances and by the oxidation of the sugar, being kept in solu-

tion. It is only when a still larger quantity of sugar is present, and has given rise to an excess of the suboxide, that it separates out. By diluting the urine its power of holding the suboxide in solution is diminished to a greater degree than the reducing power of any sugar that may be present, so that a granular precipitate may be formed when the undiluted urine gave no definite reaction. Some observers, therefore, advise a dilution of 1 in 2, or 5, in all cases before applying the test. As polyuria, with a decrease in the proportion of those substances that prevent precipitation, is a natural phenomenon in many cases of persistent dextrosuria, a definite precipitate is often formed when as little as 0·2 per cent. of sugar is present.

4. Albumen increases the copper hydroxide holding power of the urine, giving, however, a violet rather than a blue colour to the solution. It does not interfere with reduction, but prevents the precipitation of the reduced suboxide that forms, hence a small quantity of sugar may easily escape detection in an albu- minous urine. Before applying the test, therefore, the urine should be tested for albumen ; and if more than a trace (about 0·5 per cent.) is found to be present, it should be removed by acidulating with a few drops of acetic acid, boiling, and filtering.

5. Saccharin and salts of ammonia also interfere with the precipitation of the suboxide, and so their presence may prevent the detection of small quantities of sugar.

Trommer's test is not very frequently employed in this country, but it has distinct advantages over many others that are more commonly used. The reagents are stable, all the steps of the process are under control, the sources of error are evident, and the presence of disturbing influences is readily detected. More- over, the amount of sugar can be roughly estimated. Should the test readily succeed even with insufficient hydroxide saturation, the amount of sugar is large ; but should no precipitate appear, or only become evident on very accurate saturation with copper hydroxide, and more sensitive tests are found to be positive, the sugar-content of the urine is not more than 0·2 to 0·4 per cent.

To perform Trommer's test satisfactorily it is necessary that the proportions of the reagents employed should be fairly accurately adjusted to the quantity of sugar present, especially in doubtful cases, and that the mixture should not be raised to too high a temperature. It is particularly important that an excess of copper sulphate should be avoided, and more should never be added than is necessary to give rise to a few flakes of undissolved hydroxide on gently shaking, since the black oxide of copper that forms on

heating an excess may disguise the precipitate of cuprous hydrate. One part of sugar can reduce about five parts of copper hydrate, and the test should be arranged so that this proportion is, as nearly as possible, present. The cloud of earthy phosphates precipitated by the addition of the alkali to the urine often makes it difficult to decide when the necessary slight excess of copper hydroxide remains undissolved, however.

The colour of the precipitate is generally said to depend upon the alkalinity of the fluid, the brick-red cuprous oxide appearing in strongly alkaline solutions, and the yellow cuprous hydrate in solutions that are relatively only feebly alkaline. Neumayer states, however, that it is to the presence of creatinin in the urine that the formation of the amorphous yellow precipitate is due, and that the crystalline red precipitate, like that given by pure solutions of dextrose, appears when this substance is present in relatively small amounts.

Beside the reducing sugars and the normal urinary constituents already referred to, a number of substances appearing in the urine under pathological conditions, or as the result of the administration of drugs, &c., may give a more or less marked reduction with Trommer's test. Brückner gives the following list :—

1. Normal urinary constituents which, according to their presence in larger or smaller amounts, may produce varying degrees of reduction—

    Traces of carbohydrates, such as dextrose, isomaltose, pentose, animal gum, glucuronic acid compounds.

    Pyrocatechin, bile pigment, urinary pigment, uric acid, indican, creatinin, urobilin, urobilinogen.

    Concentrated urines are particularly liable to effect reduction, and the same is true of urines containing a moderate or large quantity of formed elements such as leucocytes, erythrocytes, epithelial cells, &c.

2. Products of abnormal metabolism which effect reduction :—
    Hexoses (dextrose, levulose, isomaltose, lactose), pentoses, glycogen, increased quantities of glucuronic compounds, homogentisic acid.

3. Reducing substances added to the urine as preservatives :—
    Formaldehyde, chloroform.

4. Drugs or their derivatives :—
    Acetphenetidin, antifebrin, arbutin, benzoic acid, benzosol, copaiba balsam, chloral, glucuronic acid compounds of drugs, morphin, phenacetin, saccharin, salicylic acid, salol, sulphonal, turpentine, thallin, urethan.

3. **Fehling** (**Worm-Müller**) **Test.**—Glucose cannot dissolve nearly as much copper as it can reduce, so that if the proportion of cupric hydrate in solution can be increased, the reduction is likely to be more evident with small quantities of sugar and the test be made more delicate. This is effected in Fehling's test, and the modifications of it which have been introduced, by adding tartrates, glycerine, &c., which also have the power of dissolving cupric hydrate, to the test solution, so that there may be a maximum amount of copper in solution and an optimum chance of precipitation, without the possibility of the appearance of a black deposit of cupric oxide.

In Fehling's solution Rochelle salt (potassium-sodium tartrate) is the substance employed to keep the cupric hydrate in solution.

The test solution is prepared by mixing equal parts of two liquids which may be conveniently referred to as " Fehling A," and " Fehling B." They are best prepared in the following manner (known as Soxhlet's modification).—(A) 34·64 grams of pure crystallised copper sulphate (free from iron and moisture) are dissolved in distilled water, and the solution diluted to 500 c.c. ; (B) 70 grams of sodium hydroxide of good quality (not less than 97 per cent. of NaOH), and 175 grams of recrystallised potassium-sodium tartrate, are dissolved in about 400 c.c. of distilled water, and the solution made up to 500 c.c.

Although more delicate than Trommer's test, showing 0·08 per cent. of glucose as compared with 0·25 per cent. by the latter, Fehling's has all the faults of the other test, and to a somewhat greater degree, owing to its enhanced delicacy.

Different methods of carrying out the test have been advocated by different observers, with a view to minimising the chance of error. Probably the most usual way is to bring a few cubic centimetres of Fehling's solution to the boil and then add the urine in small quantities, boiling after each addition, until reduction occurs, or an amount of urine corresponding to half the bulk of the Fehling solution employed has been added. By this method the quantity of sugar present can be roughly estimated, the larger the amount of urine required to effect reduction the lower being the percentage of sugar ; but it has the disadvantage that the prolonged boiling required when the urine is sugar-free, or only contains a trace, may bring about reduction from other causes. A second method is to add from $\frac{1}{2}$ to 1 c.c. of the urine to about 10 c.c. of boiling Fehling solution. If sugar is abundant, a yellowish or brick-red opacity and deposit are produced. If no reduction occurs, traces of sugar are tested for by adding 5 c.c. of the urine to a fresh supply

of 10 c.c. of hot Fehling, heating again to boiling, and then setting aside to cool. If no turbidity appears within a minute, the urine is considered to be free from sugar, or to contain a quantity so small as to be of no pathological importance. If the liquid loses its transparency, and passes from a clear bluish-green to an opaque lightish green, the precipitation of cuprous oxide only taking place as the mixture cools, it is probable that a small quantity of dextrose, under 0·5 per cent., is present. A third method is to boil equal parts of Fehling's solution and urine in two separate test-tubes, allow them to cool for one minute, and then pour one into the other. Any reduction that takes place within 5 to 10 minutes is then regarded as being due to a pathological amount of sugar. By this method reduction at a temperature not exceeding 60° to 70° C. is ensured, and the reducing effect of uric acid and creatinin is excluded. Another method of guarding against this source of error is to dilute the urine to a specific gravity of 1·005 (Zeehuisen), or 1·012 to 1·015 (Kellas and Wethered). The same end may also be attained by varying the quantity of the Fehling solution employed according to the specific gravity of the urine, but always using the same amount of urine (Kellas and Wethered). Thus :—

Specific Gravity of Urine.	Urine.	Fehling's Solution.
	Cubic Centimetres.	Cubic Centimetres.
up to 1·020	2	2·0
1·020–1·025	2	2·5
1·025–1·030	2	3·0
1·030–1·035	2	3·5
1·035–1·040	2	4·0
1·040–1·045	2	4·5

The mixture is boiled for a few seconds. If no precipitate forms within two minutes, it is stated that any sugar present is of no pathological significance.

At times the phosphate precipitate produced by adding the alkaline Fehling solution to a urine rich in phosphates may cause an ambiguous result. No change is observed in the cold, but on heating a fine greenish-yellow precipitate, giving an opalescent appearance to the fluid, forms. This reaction differs somewhat from the definite reduction of Fehling's solution due to traces of sugar, inasmuch as the phosphate precipitate soon becomes flocculent and separates out in more or less distinct masses. If the urine is made alkaline with sodium carbonate, the phosphates may be removed by filtration, and the filtrate will no longer give a reaction with Fehling's solution.

To avoid the effects of interfering substances when testing for small quantities of sugar a variety of other methods of removing them, or minimising their effects, have been devised. Among these may be mentioned:—

- 3 (*a*). **Allen's Method.**—In this modification of Fehling's test advantage is taken of the precipitating power of cupric acetate to remove from the urine the majority of those substances which interfere with the detection of sugar, either by themselves reducing the alkaline copper sulphate solution, retaining the cuprous oxide in solution, or producing a flocculent precipitate which masks the true reaction of the sugar.

From 7 to 8 c.c. of the urine are heated to boiling, and, without separating any precipitate of proteins that may form, 5 c.c. of the solution of copper sulphate used for preparing Fehling's solution are added, and the liquid again boiled. This produces a precipitate, principally uric acid, xanthine, hypoxanthine, and phosphates. To render the precipitation complete, however, it is desirable to add to the liquid, when partially cooled, from 1 to 2 c.c. of a saturated solution of sodium acetate, having a feebly acid reaction. The liquid is filtered, and to the filtrate, which will have a bluish-green colour, 5 c.c. of the alkaline tartrate mixture used for Fehling's solution are next added, and the mixture boiled for 15 to 20 seconds. In the presence of more than 0·25 per cent. of sugar separation of cuprous oxide occurs before the boiling-point is reached, but with smaller proportions precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate. When a urine rich in sugar is under examination the volume employed can be advantageously reduced to 2, or 3, c.c., or even less, water being added to make it up to 7 or 8 c.c. It is important that the sodium acetate should not be added until the liquid has partially cooled, in order to avoid any chance of a reaction of the resulting cupric acetate with the glucose, as in Barfoed's test.

3 (*b*). **Seegen's Method.**—This is based upon the fact that animal charcoal absorbs glucose and other reducing substances from the urine, but that those which interfere with the precipitation of cuprous oxide are retained much longer than the sugar on washing the charcoal with water.

The urine is made into a thin paste with purified animal charcoal, and left to stand for 20 or 30 minutes. The mixture is then poured on to a moist filter and the urine allowed to run through. The residue on the filter paper is now extracted with a quantity of water equal in bulk to the urine employed for the test, and when this has filtered through, the charcoal is again washed twice, with a similar quantity of water. The filtrate from each washing is kept separate and tested for sugar by Fehling's (or Trommer's) test. Seegen claims that a positive reaction with the second or third washing is absolute proof of the presence of sugar, since these washings from a normal urine will no longer reduce.

3 (*c*). **Carnelutti and Valente** recommend that 100 c.c. of the urine should be evaporated to a syrup on a water-bath, 1 c.c. of 25 per cent. solution of zinc chloride, previously mixed with a quarter of its volume of hydrochloric acid, is added, then two volumes of absolute alcohol, and the whole allowed

to stand for some hours. The liquid is filtered, the residue washed with alcohol, the alcohol evaporated from the solution, and the residual liquid made up to 100 c.c. with distilled water. Excellent results are said to be obtained with Fehling's solution by this method.

One serious disadvantage of Fehling's test is that the test solution is not very stable. Apart the "A" and "B" solutions keep fairly well, especially if they are protected from light and air ; but when once they have been mixed the mixture soon begins to deteriorate, and a more or less marked reduction will occur on merely boiling the solution with distilled water. It is therefore advisable that a control should be carried out with plain water before using a stock solution, especially if it has been made for some time. To avoid the danger of a misleading result from this cause, various modifications of Fehling's solution, in which the sodium potassium tartrate is replaced by some more stable substance, have been devised. Two only will be mentioned here.

3 (d). **Haine's Test.**—In Haine's test glycerine is the substance used to hold the cupric hydrate in solution. The test solution is prepared as follows :—

Dissolve 30 grains (1·914 grams) of pure copper sulphate, and 4 drachms (15·55 grams) of pure glycerine, in an ounce of water (28·42 c.c.) Mix this solution with 3 drachms (11·66 grams) of caustic potash dissolved in five ounces (142·1 c.c.) of water. To carry out the test, about one drachm (3·5 c.c.) of the solution is boiled in a test-tube, and from 5 to 8 drops, not more, of the urine are added. The mixture is then again boiled. In the presence of sugar a copious yellow, or yellowish-red, precipitate appears. If no precipitate forms, the urine is free from any appreciable amount of sugar. This solution has the advantage of being quite stable and keeping indefinitely.

3 (e). **Benedict's Test.**—In place of the Rochelle salt of Fehling's solution Benedict uses sodium citrate, and he also replaces the sodium hydroxide by sodium carbonate. He points out that the reducing action of glucose is dependent on the formation of a substance arising from the action of the alkali on it, and that this substance is destroyed by strong alkalies, such as caustic soda, but not by sodium carbonate. The delicacy of the test is therefore much enhanced by the use of the carbonate in place of the caustic alkali, since traces of sugar are not destroyed before they can overcome the inhibiting action of the creatinin, &c., of the urine. The test solution is prepared as follows :—

With the aid of heat dissolve 173 grams of sodium (or potassium) citrate, and 100 grams of anhydrous (or 200 grams of crystallised) sodium carbonate, in about 700 c.c. of distilled water. Filter if necessary. Dis-

solve 17·3 grams of pure crystallised copper sulphate in about 100 c.c. of distilled water, and pour slowly, with constant stirring, into the carbonate-citrate solution. Cool, and make up to 1000 c.c.

To test for sugar, 5 c.c. of the solution are placed in a test-tube, and 8 to 10 drops, not more, of the urine to be examined are added. The mixture is heated to vigorous boiling, and is kept at this temperature for one or two minutes. It is then allowed to cool spontaneously. In the presence of glucose the entire body of the solution will be filled with a precipitate, which may be red, yellow, or greenish in tinge. If the quantity of sugar is low (under 0·3 per cent.) the precipitate forms only on cooling. If no sugar is present, the solution either remains perfectly clear or shows a faint turbidity that is blue in colour, and consists of precipitated urates. The chief points to be borne in mind in using this reagent are : (1) The addition of a small quantity of urine, 8 or 10 drops, to 5 c.c. of the reagent ; this being desirable, not because large amounts of normal urine would cause reduction of the reagent, but because more delicate results are obtained by this procedure. (2) Vigorous boiling of the liquid after adding the urine, and then allowing the mixture to cool spontaneously. (3) If sugar is present, the solution, either before or after cooling, will be filled from top to bottom with a precipitate, so that the mixture becomes opaque. Since the bulk, not the colour, of the precipitate is made the basis of a positive reaction, the test can be as readily carried out by artificial light as in daylight, even when examining for very small quantities of sugar. The solution is not dark-coloured, like Fehling's solution, so that the precipitate may be readily observed without waiting for it to settle.

According to Benedict this solution is about ten times as sensitive to sugar in urine as Fehling's or Haine's solution, and, unlike these, is not appreciably reduced by creatinin, uric acid, chloroform, or the simple aldehydes, but it reacts with lactose, greatly increased amounts of glucuronic acid, homogentisic acid, &c. The solution keeps indefinitely in uncoloured glass, or cork, stoppered bottles. I have been using Benedict's solution as a routine test for sugar in my laboratory for over two years now with most satisfactory results. At first the findings were checked by other reduction methods, but these have been discarded for some time, and I now rely on it alone as a preliminary test in all cases.

4. Almén-Nylander's (modified Böttger's) Bismuth Test.—

In the original bismuth test, as described by Böttger, the urine was heated to boiling with sodium hydrate and a small pinch of basic bismuth nitrate. As it was found that alterations in the alkalinity of the fluid controlled the results to a certain extent, Almén and Nylander worked out a test solution which would give more constant and reliable findings.

This is prepared by dissolving 4 grams of potassium-sodium tartrate in 100 c.c. of a 10 per cent. solution of sodium hydroxide (sp. gr. at 19° C. 1·115) with gentle heat, and then saturating with bismuth subnitrate (about 2 grams are necessary). After cooling the solution is filtered through glass-wool, and kept in a dark-coloured bottle away from the light. Preserved in this way the reagent is permanent for years.

To carry out the test, about one-tenth of its volume of the reagent is added to a specimen of urine, and the mixture boiled for from two to five minutes. The fluid may be prevented from boiling over by introducing a coil of platinum wire, or it may be heated in a boiling water-bath. If sugar is present the solution turns black, and a black precipitate of bismuth settles out. Where there is over 0·2 per cent. of sugar the yellow colour of Moore's test is first seen. If there is no sugar, a white precipitate of phosphates only will appear. A very small trace of sugar will merely turn the fluid brown, although it may appear black by transmitted light, and will tinge the phosphate deposit a slight grey—a change which is more marked on the upper surface than in the depths of the deposit after it has settled. If no change is observed after two minutes' boiling, the full five minutes should be allowed, as a sudden darkening may take place in a urine that has previously shown no change. It is most important that the reagent should be accurately one-tenth of the volume of the urine if only traces of sugar are to be detected. If the solution only turns dark on cooling, the test is not positive.

This test is useful as a confirmation of Trommer's, or Fehling's. It is not affected by most of the more important disturbing substances which interfere with the reliability of those tests, and gives no reaction with normal urines, yet it is delicate enough to show 0·025 per cent. of sugar. Concentrated urines may, however, give a positive reaction. A considerable amount of combined glucuronic acid will give a reaction, and the reduction that occurs after the ingestion of senna, rhubarb, eucalyptus, kairin, quinine in large doses, and oil of turpentine, probably depends upon the presence of this substance. With rhubarb and senna the fluid is brownish-red from the action of the alkali. Uroerythrin and

hæmatoporphyrin may give deceptive results, tinging the phosphate deposit a dark brown. If the urine is ammoniacal the action of the test may be interfered with, as part of the sodium hydrate is consumed in replacing the ammonia, leaving the solution insufficiently alkaline. One of the most important disturbing substances is albumen. This produces a precipitate of sulphide of bismuth which, with small quantities (0·6 per cent.), may be distinguished by its reddish-brown colour; but large amounts of protein (1·2 per cent.) yield a brownish-black precipitate which is easily confounded with that due to sugar. All albumen should therefore be removed before applying the test. The sulphur-containing compound present in the urine after eating asparagus also yields a similar precipitate, and is a fruitful source of error.

According to Brückner the disturbing influences with this test may be classified as follows :—

(1) Normal urinary constituents which, according to their presence in larger or smaller amounts, may produce varying degrees of reduction, or change, in colour of the earthy phosphates : uroerythrin and urinary pigments (urobilin particularly) when present in greatly increased quantities cause a brownish discoloration of the phosphate deposit, indican.

(2) Products of abnormal metabolism which effect reduction : hexoses (dextrose, levulose, isomaltose, lactose), pentoses, glycogen, increased quantities of glucuronic acid compounds, blood pigments, increased quantities of hæmatoporphyrin, homogentisic acid (only in concentrated solution).

(3) Drugs, or derivatives from them as the result of metabolic changes : antipyrin, arbutin, benzoic acid, benzosol, large doses of quinine, chloral, eucalyptol, glucuronic acid compounds of drugs, indican, kairin, rheum, frangula, cascara sagrada, salol, senna, sulphonal, turpentine, trional.

(4) Substances which influence the sugar reaction : ammonium carbonate, albumen in considerable amounts.

Modifications of the bismuth test have been devised by Brücke and Maschke, but, as they possess no striking advantage, it is not necessary to refer to them further.

5. **Mercury Tests.**—Alkaline solutions of mercury salts are reduced by the sugars, giving a grey deposit of mercury, but as they are almost exclusively used for quantitative work they will be considered under that heading. (See Sachsse's and Knapp's

methods.) They are open to the same fallacies as copper solutions, and have no compensating advantages.

6. Picric Acid (Braun).—Picric acid (*Trinitro-phenol*) was at one time much used as a test for sugar, and its advantages were strongly insisted upon by Sir G. Johnson.

The test is carried out by mixing equal volumes of the suspected urine and a saturated solution of picric acid, and then adding one-fourth the volume of a 6 per cent. solution of potassium hydrate (*Liq. potassæ*). If much sugar is present, a well-marked orange-red colour develops. On boiling the mixture the colour deepens, the extent of the change depending upon the sugar-content of the urine. If there is a considerable amount the liquid becomes an intense brownish-red, so deep as to be almost opaque ; but with small quantities the colour is a cherry-red, which it is not easy to distinguish from the similar coloration given by many normal urines. It is advisable to compare the result with a control carried out with a urine known to be free from sugar. Allen states that a serviceable and permanent reagent may be prepared by mixing two volumes of a cold saturated solution of picric acid with one volume of a normal caustic soda solution (4 per cent.), disregarding any crystals that separate out.

Picric acid gives no reaction with uric acid, urates, and mucin ; but with creatinin, creatin, and glucuronic acid it gives a very similar reaction to glucose. Normal urines, therefore, yield a red coloration with an alkaline solution of picric acid, even in the cold, and this is intensified by boiling, so that it is difficult to be sure of the presence of traces of sugar. If the creatinin is removed by treating the urine with 25 per cent. of its volume of a cold saturated solution of mercuric chloride, and 5 per cent. of a cold saturated solution of sodium acetate, boiling for five minutes and filtering hot, acidulating the filtrate with acetic acid, boiling for ten minutes with zinc dust, and again filtering to remove the mercury, the picric acid test will indicate very small quantities of glucose.

7. Indigo Test (Hoppe-Seyler).—An alkaline solution of ortho-nitro-phenylpropiolic acid is reduced on heating with a solution of glucose to indigo blue.

The test solution is prepared by dissolving 5·76 grams of the acid in 100 c.c. of 10 per cent. sodium hydrate solution, and diluting to one litre with water.

Five cubic centimetres of this solution are heated with ten drops of urine for quarter to half a minute when, if at least 0·5 per cent.

of sugar is present, a blue coloration is seen. A high percentage of sugar may, however, cause the indigo-blue to be still further reduced to indigo-white, but on shaking the fluid with air it will give a blue foam. Traces of indigo may be detected by shaking the solution with chloroform, which will give a blue solution if indigo is present. It is important that not more than ten drops of the urine should be used, as it is found that a larger amount (20 drops) will give a slight reaction with normal urines. Concentrated urines should be diluted before applying the test.

It is said that no other substances than the sugar commonly met with in the urine give any reaction if the test is correctly and carefully carried out. Over 2 per cent. of albumen causes the solution to assume a dark-red colour.

A solution of *indigotin-disulphonic* acid (Mulder) saturated with sodium carbonate, and boiled with the urine, will turn successively green, purple, red, and yellow if sugar is present. On shaking the warm solution the colour changes are reversed. Glucuronic acid, inosite, gallic, tannic, and salicylic acids and their compounds will give a similar reaction.

8. Aniline Dye Tests.—Various coal-tar dyes are decolorised on being heated with alkaline solutions of dextrose and other reducing agents. Wender, Crismer, and others have utilised this fact for the recognition, and estimation, of sugar in the urine. The former employed methylene blue, and the latter safranin.

8 (*a*). **Methylene Blue Reaction (Wender).**—A solution of methylene blue is prepared by dissolving 1 gram in 3000 c.c. of distilled water. Six cubic centimetres of this solution are mixed with 2 c.c. of a normal solution of caustic potash (5·6 per cent.). The urine is diluted ten times with water, and 2 c.c. of the dilution are added to the alkaline solution of methylene blue. The mixture is then boiled for one or two minutes, avoiding agitation, or contact with the air, as much as possible. If the urine contains 0·5 per cent., or more, of sugar the blue colour will be completely discharged. By using different proportions of urine, and heating in a series of test-tubes, in a water-bath, an indication of the amount of sugar present may be obtained.

8 (*b*). **Safranin Test (Crismer).**—Two cubic centimetres of solution of safranin, made by dissolving 1 gram of the dye in a litre of distilled water, are mixed with 2 c.c. of a normal solution of caustic soda (4 per cent), or caustic potash (5·6 per cent.), and 2 c.c. of the urine added. The mixture is then boiled. If glucose is present to the extent of 0·1 per cent. the red is changed to a pale

yellow colour, and the liquid becomes turbid from the separation of the insoluble leuco-derivatives. If the sugar is not present in considerable excess the red colour returns on agitating the liquid, or exposing it to the air.

Urines containing a high percentage of sugar should be well diluted before applying the test. Safranin in alkaline solutions is not decolorised when heated by creatinin, creatin, uric acid, urates, chloral, chloroform, hydrogen peroxide, or salts of hydroxylamine, or by mucin. It is only slowly affected by albumen.

The great objection to the safranin, and the methylene blue, tests is that they are so sensitive that normal urines, as a rule, give a distinct reaction. Kellas and Wethered found that with the safranin test a reduction corresponding to 0·07 to 0·08 per cent. of glucose was given by normal urines. They consider, however, that, in spite of this drawback, it is the most convenient and reliable test for sugar that can be applied in the present state of our knowledge, especially if a reduction due to the presence of glucuronic acid is excluded in doubtful cases, and that, used as an auxiliary to Fehling's test, it is sufficient to settle many troublesome cases where small quantities of sugar, and large quantities of creatinin, cause the findings of the latter to be uncertain.

9. **Phenylhydrazin Test (Fischer, v. Jaksch).**—The application of phenylhydrazin as a reagent for the detection of sugar in the urine marked an epoch in the investigation of glucosuria and allied conditions, for not only is it more delicate, showing 0·01 per cent. of sugar, than any test previously employed, but it is unaffected by other substances, such as creatin, creatinin, hippuric acid, homogentisic acid, and excess of uric acid and urates as met with in human urine, that are liable to give rise to difficulties when most other methods are relied upon. So delicate is it that, theoretically, the small amount of sugar in normal urine should give a reaction, but practically this is not found to be the case, unless a special technique is followed. Zunz states that osazone crystals are not found unless the urine also reduces Fehling's solution to some extent. On the other hand, the test may fail even when the urine is known to contain sugar, if it is not carefully carried out. Success depends chiefly (1) upon the purity of the phenylhydrazin, hence the more stable hydrochloride is generally preferred ; (2) the amounts of the reagents used, theoretically 1 part of sugar, 2 of phenylhydrazin, and 3 of sodium acetate are best ; and (3) the time allowed to cool. Even under the most favourable circumstances all the sugar is not precipitated. From a 5 per cent. solution of

glucose, Fischer found that the maximum precipitate represented from 85 to 90 per cent. of the sugar in the solution. Since albumen interferes with the separation of the crystals, it should be removed before applying the test.

The method of performing the phenylhydrazin test described by von Jaksch has been much modified by subsequent writers, and, since the physical conditions under which it is carried out materially affect the result, it will be necessary to refer to the chief variations proposed.

9 (a). Von Jaksch originally recommended that 50 c.c. of the urine to be tested should be mixed with 2 grams of sodium acetate, and from 1 to 2 grams of phenylhydrazin hydrochloride, and that the mixture be heated in a water-bath for twenty minutes to half an hour. If glucose is present, the osazone then separates out, on cooling, as an amorphous, or crystalline, deposit of a yellow or reddish colour. If amorphous, the precipitate can be recovered in a crystalline form by filtering it off, washing with distilled water, dissolving the residue on the filter in hot 50 per cent. alcohol, diluting the solution with water, boiling to expel the alcohol, and cooling.

9 (b). Two drops of a concentrated solution of lead acetate are added to 10 c.c. of the urine, and the precipitate filtered off. One drop of acetic acid, or enough to acidify the filtrate, a piece of phenylhydrazin hydrochloride the size of a pea, and sodium acetate the size of a bean, are then added, and the mixture boiled on a water-bath for from one to two hours. It is then filtered hot, returned to the water-bath, and allowed to cool slowly.

9 (c). These methods of performing the test take time, and require the use of special apparatus which is not always available. To obviate these difficulties, and render the reaction convenient for clinical use, it has been suggested that 0·5 grams of phenyl-hydrazin hydrochloride, and 1·5 grams of sodium acetate, should be dissolved by gentle heat in a few cubic centimetres of water in a test-tube, and then 5 to 10 c.c. of the urine added. The mixture is brought to the boiling-point, and maintained there for three minutes with strong, and five minutes with weak, solutions of sugar. The test-tube is then set aside to cool, and the deposit examined for osazone crystals in five or ten minutes. In my experience this rapid method of performing the phenylhydrazin test gives a re-action with all sugars when 10 c.c. of the urine is used, and the heating continued for at least five minutes. In the water-bath, however, even an hour is not sufficient to obtain a satisfactory yield with maltose, lactose, and pentose, an hour and a half, or

even two hours, being required to demonstrate their presence satisfactorily, especially when the solution is weak.

9 ·(d). Some authorities, following E. Fischer, have preferred to use phenylhydrazin and not the hydrochloride, but it has the disadvantage of not keeping well. It should be almost straw-coloured, and is conveniently kept in sealed bottles containing only a small quantity, which can be quickly used when once opened. A knife-point of sodium acetate is added to 10 c.c. of the urine, then 1 to 2 c.c. of 10 per cent. acetic acid, and 5 drops of pure phenyl-hydrazin. The mixture is heated in the water-bath, or over the free flame, in the same way as when the hydrochloride is employed.

9 (e). A modification of this method, suggested by Kowarski, which gives very satisfactory results, and is more delicate, consists in mixing 5 drops of pure phenylhydrazin in a test-tube with 10 drops of acetic acid, gently shaking. and then adding about 1 c.c. of a saturated solution of sodium chloride. To the solid mass that forms is added 3 to 5 c.c. of the urine, and the test-tube is then heated, in the free flame, for two minutes after its contents begin to boil. On cooling, the osazone crystals separate from urines containing over 0·2 per cent. of sugar in one minute, and from weaker solutions in about five minutes.

Beside giving a crystalline osazone with the sugars dextrose, levulose, lactose, maltose, isomaltose, and the pentoses (arabinose and xylose) met with in the urine, phenylhydrazin also forms a compound with glucuronic acid, and exceptionally with acetone, aceto-acetic acid, oxalic acid, and in very concentrated urines with uric acid. As human urine is relatively poor in uric acid, the last named does not call for further remark. The acetone compound occurs as needles which melt to oily globules at 16° C., while the oxalic acid salt separates as insoluble, glancing, colour-less plates which melt at 172° to 173° C.

Alkaline salts of glucuronic acid form a compound with phenyl-hydrazin, which slowly separates on cooling as yellow needles, usually of a somewhat darker colour, of smaller size, and more irregular shape, than the osazones of the sugars. Although the deposit is small, it may be easily mistaken for traces of sugar compounds, and so give rise to an incorrect diagnosis. It is this mistake that has to be chiefly guarded against in using the phenyl-hydrazin test for diagnostic purposes. Some compounds of glucu-ronic acid undergo the decomposition that must occur before it combines with phenylhydrazin more easily than others, so that the readiness with which the reaction takes place varies. A urine containing a compound such as urocholic acid, which readily

splits up, quickly responds to the test ; but the more resistant
phenol and indol compounds require prolonged treatment before
they react. When the reaction takes place in a solution containing
a free acid, the phenylhydrazin compound is precipitated in the
form of dark-brown granules, and does not assume the crystalline
form. It has been stated that prolonged heating tends to cause
the glucuronic acid compound to be precipitated on cooling as a
brown amorphous mass and not in the crystalline form (Purdy),
which is liable to be mistaken for the osazone of a sugar. In the
examination of a hundred normal urines I sought to test the truth
of this statement, and found that, when heated in the water-bath
for an hour, four of them showed a crystalline deposit, while by
boiling in the free flame for five minutes, six specimens gave a
positive result. Using the same methods, but shortening the period
of heating to twenty minutes and two minutes respectively, exactly
the same results were obtained. So that the mere time, or method,
of applying heat cannot be relied upon to differentiate glucuronic
acid from the sugars. In practice the phenylhydrazin compounds
of glucuronic acid and the sugars can only be differentiated by
experience of the different appearance of their crystals, which is,
however, a somewhat fallacious guide, and by a consideration of
their physical and chemical characters. These will be fully dealt
with when the classifying tests are considered.

9 (*f*). A modification of the phenylhydrazin test, which is useful
when no microscope is at hand to examine the osazone crystals,
is carried out as follows :—

Riegler's Modification.—0·1 grams of phenylhydrazin hydrochloride, and
0·5 grams of sodium acetate, are dissolved in a few drops of water, and
boiled for several minutes with 1 c.c. of the urine ; 1 c.c. of caustic soda
(**10** per cent.) is then added. If sugar is present a deep red-violet colour
should appear within five minutes. The test is best carried out in a
porcelain dish, as the colour changes are better seen, and appear as rings,
or streaks, that are very striking. The test is said to react with a solution
containing 0·01 per cent. of glucose. Other sugars, and formaldehyde, also
give the reaction. As the solution turns rose-red from oxidation on standing
for an hour or so, it is essential that a distinct colour reaction should be ob-
served in five, or at most ten, minutes after the addition of the soda solution.

II. *Classifying Tests*

1. **Fermentation.**—The fermentation test serves to distinguish
the fermentable sugars from those reducing substances that are
not attacked, or only with difficulty, by ordinary brewer's yeast.
It is important, however, that a time limit of six to twelve hours,
should be set in which fermentation at a definite temperature

(34° to 36° C.) should occur, as otherwise gas may appear as a result of other causes and lead to a mistaken diagnosis. The test is performed as follows :—

A piece of compressed yeast, about the size of a large pea, is rubbed up with 25 to 30 c.c. of the urine and the mixture poured into a test-tube until it is filled to the brim. The tube is then closed with a perforated cork which carries a V-shaped piece of glass tubing, and is placed in a beaker and kept at a temperature of 34° to 36° C. in the incubator (or a properly constructed fermentation apparatus may be used). If the urine contains a fermentable sugar, gas will accumulate in the upper end of the tube and expel a corresponding amount of urine into the beaker. To prove that the gas is carbon dioxide, the cork should be removed from the end of the tube under mercury or water, and a small quantity of caustic soda introduced. It will then be seen that, owing to the absorption of the gas, the liquid will again rise to the top of the tube. The presence of alcohol may be shown by distilling the urine and testing the distillate with iodine and caustic potash for iodoform. Two control tests should always be carried out : (1) One with a normal urine, to which a little dextrose and yeast have been added, to prove the activity of the yeast ; (2) another with normal urine and the yeast alone, to show that there is no gas formation apart from fermentation. The last test is essential, as compressed yeast often develops gas with normal specimens, particularly if the urine is only faintly acid and the time of fermentation is prolonged. This is due to ammoniacal changes brought about by bacteria contaminating the yeast, which develop carbonic acid from the ammonium carbonate derived from the urea. The ammoniacal fermentation is slower in its development than the alcoholic fermentation of the sugars, and can be suppressed by boiling the urine to sterilise it. Boiling also frees it from air, which is another possible source of fallacy. Some prefer to add 1 per cent. sodium fluoride to the urine, or to render it distinctly acid with tartaric acid, boil for a few minutes, and then cool, before applying the test. Some samples of yeast contain traces of sugar, and give rise to gas formation from the fermentation of this. By washing the yeast the sugar may be removed, and its presence proved by the washings responding to the tests for sugar. As a rule, however, compressed yeast is free from sugar. Certain samples of yeast give rise to gas formation by what is termed " self-fermentation," a process apparently related to the amount of contained glycogen. Both these sources of error are detected by carrying out a control test.

By this test the sugars and other reducing substances occurring in the urine may be divided into the following groups :—

(1) Those that are quickly fermented with brewer's yeast (6 to 12 hours) : dextrose, levulose, maltose.

(2) Those that are slowly broken down and fermented (20 to 30 hours) : cane-sugar, lactose, galactose, isomaltose.

(3) Those that show no gas formation (in 20 to 30 hours) : pentoses, glucuronic acid, laiose, dextrin, glycogen, homogentisic acid, inosite, uric acid, creatin, and creatinin.

2. **The Polariscope.**—The specific rotatory powers of the sugars, and related reducing substances, occurring in the urine divides them into three classes :—

(1) Those that are dextro-rotatory (dextrose, galactose, lactose, maltose, isomaltose, and l-arabinose).

(2) Those that are levo-rotatory (levulose, laiose, and most compound glucuronates).

(3) Those that are optically inactive (i-arabinose).

A determination of the specific rotatory power of the urine, especially when this is carried out quantitatively and is compared with its reducing power, helps in the detection of the particular variety of reducing substances present. In the recognition of traces of sugar care must be exercised, since normal urines are slightly levo-rotatory (about $0·05°$ to $0·17°$), and occasionally urines are dextro-rotatory when sugar is absent from the presence of glucuronic acid compounds (Borntrager in two morphia habitués). The presence of albumen interferes with the recognition of sugars by the polariscope, since it is levo-rotatory, and hence may cover a slight dextro-rotation due to that cause. Cystin, oxybutyric acid, and most paired glucuronates are also levo-rotatory and may have a similar effect. All these substances are not fermented by yeast, so that the polarimetric reading due to their presence is the same after as before fermentation ; but if a urine contains a fermentable sugar the reading is lowered by fermentation. When a urine contains both a dextro-rotatory and levo-rotatory fermentable sugar (*e.g.* dextrose and levulose), and these alone, the polarimetric estimation will give a smaller value than that obtained by titration, and when it is fermented its rotatory power should be nil, or only equal to that of a normal urine. If, however, there is also an unfermentable levo-rotatory substance, such as β-oxybutyric acid, present, not only will the polarimetric and titration readings not agree, but the urine will still be levo-rotatory after fermentation, but this will not be sufficient to entirely account for the different results obtained with the polariscope and by titration. The presence of paired glucuronic acid may have a similar effect. The pentose usually met with in the urine in chronic pentosuria is optically inactive (i-arabinose), but Luzzato has described dextro-rotatory l-arabinose as being present in one case. The latter is also met with in alimentary pentosuria. Since the degree of polarisation induced by maltose is much more intense than that produced by dextrose, a small quantity of the former may give the same reading as a much larger quantity of the latter ; but the reducing power of maltose is increased by hydrolysis with

a dilute acid, while that of glucose is unchanged, or even diminished, from the formation of human substances.

3. Barfoed's Test.—When carried out under certain conditions this test serves to distinguish (1) the monosaccharides (dextrose, levulose, and galactose) from (2) the reducing disaccharides (lactose and maltose).

From five to fifteen drops of the urine are mixed with 5 c.c. of the test solution (made by dissolving 13·3 grams of crystallised neutral copper acetate in 200 c.c. of 1 per cent. acetic acid), and boiled in a water-bath for from 3 to 5 minutes. The members of the first group will reduce the solution, giving a yellow or red precipitate within the times mentioned, but the disaccharides cause no change.

4. Phloroglucin Test.—This test is chiefly used to detect the presence of pentoses, but it is also given by glucuronic acid, and, as regards the colour change, by lactose and galactose. It is therefore not specific, but is merely a classifying test, helping to distinguish these substances from the other sugars.

According to Wheeler and Tollens it is carried out as follows. To a few cubic centimetres of the urine are added an equal quantity of fuming hydrochloric acid (sp. gr. 1·19), and from 25 to 30 milligrams of phloroglucin. The solution is warmed until a red colour develops. On examination with the spectroscope the presence of a pentose, or glucuronic acid, is shown by the appearance of a band between D and E (yellow and green). If this is not found the solution is brought to the boil and again examined with the spectroscope. Lactose and galactose give the red coloration, but do not show the band on spectroscopic examination. Normal urines frequently give a doubtful reaction from the presence of glucuronic acid.

Salkowski recommends the following modification. Five or six cubic centimetres of fuming hydrochloric acid are warmed and saturated with phloroglucin, leaving a little undissolved. This solution is divided into two parts. To one-half is added 0·5 c.c. of the urine to be examined, and to the other the same amount of a normal urine. Both are placed in a beaker of boiling water. A positive reaction is shown by the appearance of an intense red colour, which begins above and extends downward, in the mixture containing the suspected urine, while the control exhibits no marked colour change. Examination with the spectroscope gives the same result as in the preceding method. The colour change is better seen if the urines are decolorised by being warmed with animal charcoal, and filtered, before commencing the test. The specimens should be removed from the water-bath as soon as the colour has developed distinctly, as prolonged heating interferes with the clearness of the reaction. If direct examination of the liquid with the spectroscope is negative the solution should be extracted with amyl alcohol, and this extract be examined spectroscopically.

5. **Physical and Chemical Characters of the Phenylosazones.**—The phenylhydrazin compounds of the sugars possess certain physical and chemical characters by which they can be more or less readily differentiated.

The chief of these are : (1) The rate of osazone formation, (2) the microscopical characters of the crystals ; (3) the solubilities of the osazones in various reagents ; (4) the specific rotatory power of these solutions ; (5) the melting-points of the purified products ; (6) their percentage content of nitrogen.

1. *The rate of osazone formation* is a point of considerable importance in determining the variety of sugar present in a particular solution. Under experimental conditions, with solutions of definite strength, exact time limits for the appearance of the osazone can be laid down. Although this is not feasible with such a liquid as urine, where the proportion of sugar present is unknown, valuable information can be obtained by observing the conditions and rate of osazone formation. The compound formed by dextrose and levulose separates from the hot solution after a comparatively brief interval, the former in most cases with characteristic suddenness. The osazones of maltose, lactose, and the pentoses only form after much more prolonged heating, and although crystals of pentosazone may eventually separate from the hot solution, maltosazone and lactosazone never appear until the fluid cools, no matter how long it may be boiled.

2. *Microscopical examination* of the crystalline deposit obtained after treatment with phenylhydrazin shows certain differences in the characters, size, and arrangement of the crystals which are suggestive. The large yellow needles arranged in sheaves and rosettes yielded by dextrose are well known. Levulosazone resembles glucosazone, but there is less tendency to rosette formation, and the crystals are somewhat longer and more slender. Lactosazone occurs as spherical masses of crystals resembling a shaggy yellow chrysanthemum. At the periphery the separate crystals can be distinguished, and are seen to be slender, flexible, and hair-like, but in the centre they are felted together into a brown semi-opaque mass. In preparations made by the rapid method the individual crystals are usually not as distinct as those formed after prolonged heating in the water-bath, the appearance presented being that of a brown central boss surrounded by a light yellow halo showing " fine " radial striations. The crystals of maltosazone are short, stiff, and sword-like, and are arranged in small rosettes when prepared by the water-bath method. Preparations made by heating in the free flame are less characteristic,

consisting of narrow crystals grouped in small bushy sheaves. Isomaltose yields masses of aggregated flexible needles of a golden-yellow colour, mostly arranged in spheres. The pentose crystals are silky, tangled, curved needles, generally arranged in rosettes. The shape, size, and arrangement of the crystals is influenced to a certain extent by the manner in which the test is carried out, and by the rate of cooling, so that not only is some experience of typical preparations required in forming an opinion, but the physical conditions under which the sample under examination was prepared must also be taken into account. In some cases the crystalline form can only be determined satisfactorily after the specimen has been purified by recrystallisation from alcohol, boiling acetone, pyridin, &c.

3. The osazones can be differentiated to a certain extent by their *solubilities*. Thus pentosazone is readily soluble in water at 60° C., but dextrosazone is almost insoluble. One part of lactosazone dissolves in eighty to ninety of boiling water, while maltosazone is still more soluble (1 in 70 to 75). Isomaltosazone dissolves one part in four of water at 100° C.

Dextrosazone is only very slightly soluble in cold methyl alcohol, levulosazone is a little more soluble, but the pentosazones and glucuronic acid compound readily dissolve. All the osazones are soluble in hot 50 per cent. alcohol, and advantage is taken of this fact to prepare them in a pure form.

I have found that the rate of solution of the osazones in dilute sulphuric acid is of some value in distinguishing the phenylhydrazin compounds of dextrose and levulose from those of other carbohydrates. On irrigating a preparation of the latter with a 33 per cent. solution of the acid, the crystals turn brown and dissolve rapidly, while dextrosazone and levulosazone only slowly assume a brown coloration and take several minutes before they disappear.

4. *Specific Rotatory Power.*—When a solution of 0·2 gram of the purified osazone is dissolved in 4 grams of pyridine and 6 grams of absolute alcohol and examined with the polariscope in a 100 mm. tube, the nature and degree of rotation is found to vary with the sugar.

Three are dextro-rotatory	.	.	l-arabinose (+1·1°), galactose (+0·48°), maltose (+1·3°).
Four are levo-rotatory	.	.	l-xylose (−0·5°), dextrose (−1·3°), levulose (−1·3°), and mannose (−1·3°).
One is inactive	.	.	lactose (±0·00°).

Solutions of dextrosazone, maltosazone, &c., in glacial acetic

acid are levo-rotatory, but a solution of galactosazone is optically inactive when it contains less than 4 per cent. ; over that amount it is faintly levo-rotatory.

Examination of the crystals mounted in the mother liquor, with polarised light, under the microscope, also helps to distinguish the osazones of the reducing sugars from glucuronic acid crystals, for the former stand out bright, and appear green, red, &c., while the latter are dark and uncoloured.

5. The *melting-point* of the crystals obtained from urine by the phenylhydrazin reaction is one of their most useful and characteristic properties. The product employed for the purpose must, however, be pure, or doubtful and misleading results will follow. Thus the melting-point of pure dextrosazone is 204° to 205° C., but the impure crystals obtained direct from the urine generally melt somewhere between 173° and 194° C. Levulosazone melts at the same temperature as dextrosazone. The melting-point of the phenylhydrazin compound of lactose is about 210° C., and of maltose 206° to 207° C. Pure galactosazone melts at 194° to 197° C., but when separated from the urine, at 171° to 174° C. Isomaltose begins to form drops at 140° to 145° C., melts at 150° to 153° C., and blackens at 200° C. The osazone of l-arabinose, in a pure form, melts at 160° C., and r-arabinose at 166° to 168° C., but as obtained from the urine the melting-point lies between 156° and 158° C. As the osazones undergo decomposition on prolonged heating, it is necessary that the temperature should be rapidly raised at first, and then gradually increased as the point of fusion is approached, or charring of the specimen may obscure the change of state.

6. The formula of the osazones formed by the monosaccharides dextrose and levulose is $C_{18}H_{22}N_4O_4$, while that of the dissaccharides lactose, maltose, and isomaltose is $C_{24}H_{32}N_4O_9$. Hence the former may be expected to yield 15·64 per cent., and the latter 10·76 per cent. of nitrogen. In practice slightly lower readings are found to be the rule, about 15·58 per cent. being obtained for dextrose and levulose, and 10·67 per cent., or thereabout, for the disaccharides. The percentage of nitrogen contained in the pentosazones is 17·07, as the formula is $C_{17}H_{20}N_4O_3$. By determining the percentage of nitrogen in an osazone it is therefore possible to decide to which of these three classes of carbohydrate the sugar belongs. A satisfactory determination is, however, only possible when a sufficient amount of the pure product and the necessary apparatus are available for a combustion experiment, as Kjeldahl's process is useless for the purpose, the separation of the nitrogen not being complete.

III. *Confirmatory or Special Tests*

In the preceding pages the means by which sugars, and other reducing substances, occurring in the urine can be detected, and the tests by which these can be classified, have been described. A careful consideration of the results of the classifying tests in any particular instance will have indicated which particular substance, giving the tests of the first group, is probably present. The special or confirmatory tests by which a definite diagnosis can be made now remain to be dealt with.

Most of the confirmatory reactions, although more or less specific, are not absolute, and much depends on the way that they are carried out. In some instances, too, a satisfactory result is only obtained when the sugar, or other reducing substance, has been isolated from the urine and the test is applied to a pure solution. We shall therefore first briefly describe the methods usually employed for *isolating sugars* from the urine.

A. When the urine contains from 5 to 10 per cent. of dextrose mere evaporation on a water-bath, to the consistency of a syrup, will often cause it to separate out in tabular crystals, or irregular warty masses, on cooling and standing for some days. More frequently it is deposited in crystals consisting of a compound of dextrose with sodium chloride, which is more soluble in water, but less soluble in alcohol than glucose itself. Sometimes, however, the sugar will not separate out, even when the syrup is left at rest for many days. In such cases treatment with ether, about half the volume of the syrup, which is subsequently allowed to evaporate spontaneously, will induce the separation of the crystals. The syrup, and any glucose crystals that it may contain, are filtered off, and purified from urea and extractive matter by treatment with a small amount of cold absolute alcohol, which leaves most of the sugar undissolved. The residue is then boiled with absolute alcohol, which dissolves the sugar and leaves an insoluble residue of sulphates, phosphates, and urates. The hot alcohol extract is filtered, and evaporated to a small bulk. On cooling crystals of dextrose separate out. These may be purified by recrystallisation from methyl-alcohol. Levulose can be separated from dextrose by treating the syrup with lime, with which the former forms an insoluble compound. This can be separated from the soluble dextrose compound by filtration, washed, and decomposed with oxalic acid.

B. Precipitation by metallic salts.

(*a*) *Lead*.—Carbohydrates, and most of the oxidation, and reduction products of carbohydrates, form compounds with lead by which they may be isolated, and by a process of fractional precipitation it is possible to separate them to a certain extent. The separation, however, is not quite sharp, particularly in mixtures and impure solutions,

D

such as urine. For the isolation of small quantities of sugar from the urine the following procedure may be adopted (Brücke, Pavy). A large quantity of the urine is treated with half its volume of a 10 per cent. solution of neutral lead acetate. The precipitate that forms (1) is filtered off, and the filtrate treated with basic lead acetate, any further precipitate being also filtered off (2). The filtrate from this is treated with ammonia and a further supply of basic lead acetate, unless a distinct excess of the latter has been already used. The precipitate that forms (3) is filtered off, and the filtrate again treated with basic lead acetate and ammonia. Any precipitate that forms is separated by filtration (4). The precipitate (1) contains the urates, uric acid, xanthin, sulphates, phosphates, and colouring matter, beside glycogen and part of any levulose that may be present. The precipitate produced by basic lead acetate in the acid urine (2) contains glucuronic acid, laiose, and glycogen. The third (3) and fourth (4) precipitates contain the sugars (dextrose, levulose, maltose), beside some glucuronic acid and laiose which were not completely precipitated in the acid solution. Galactose and lactose are only incompletely precipitated, even by basic lead acetate in the presence of ammonia. To separate the sugars, &c., from their combination with lead, the mixed third and fourth precipitates are washed with distilled water until the wash water is neutral, or only faintly alkaline. The residue is then suspended in water and decomposed with a stream of sulphuretted hydrogen (sulphuric, or oxalic acid, may also be used, but the product is more highly coloured). The liquid is now cautiously treated with sodium carbonate until just neutral, when the colouring matter separates out and may be filtered off. Further decolorisation may be effected by slightly acidifying the liquid with acetic acid, and shaking with a little animal charcoal (previously freed from phosphates, &c., by boiling with hydrochloric acid and washing until the washings are no longer acid to litmus). The sugars may be recovered from this liquid by evaporating and crystallising, or the solution itself may be used for the necessary tests.

To separate *lactose* and *galactose* from the urine, it is saturated with lead acetate and filtered, the filtrate treated with ammonia, and the precipitate that forms washed with distilled water. The filtrate, and wash water, are again treated with lead acetate and ammonia, and the process repeated until a filtrate is obtained that is no longer dextro-rotatory. Any resulting precipitates are added to that first obtained. The combined precipitates are suspended in water and treated with a stream of sulphuretted hydrogen. The lead sulphide is filtered off, the optionally active filtrate is shaken with silver oxide, filtered, and the dissolved silver removed with sulphuretted hydrogen. The filtrate from this is evaporated, in the presence of barium carbonate, to a small volume, and filtered. It is then treated with 90 per cent. alcohol and the flocculent precipitate that forms filtered off. The sugar is crystallised out from the filtrate over sulphuric acid, and the crystals purified by dissolving them in water, decolorising with animal charcoal, and re-

crystallising. A fresh crop of crystals may be obtained from the mother liquor by a further addition of alcohol.

(b) *Copper*.—According to Salkowski the sugars, and particularly glucose, can be precipitated by copper sulphate and an alkali, forming an insoluble blue or green double compound. The formation of insoluble copper compounds is most commonly employed for the separation of the carbohydrates from protein substances, and for this purpose copper acetate, or chloride, is generally employed. The neutral solution to be examined is mixed with a large amount of a concentrated solution of copper chloride and any precipitate that forms is filtered off. To the filtrate is then added sodium hydrate in sufficient quantity to combine with all the hydrochloric acid of the copper chloride used, and also give two molecules for each molecule of copper chloride. The precipitate is filtered off and well washed with hot water. As some copper compound of the carbohydrate still remains in solution, the filtrate is mixed with a large excess of alcohol. The precipitate that forms is filtered off, and washed with alcohol containing sodium hydrate in solution, till the filtrate no longer gives the biuret reaction. The combined precipitates are dissolved in dilute hydrochloric acid, and re-precipitated by the calculated amount of alkali. The purified precipitate is suspended in water, the copper precipitated by a stream of sulphuretted hydrogen and the filtrate evaporated down *in vacuo*. The sugar is then crystallised out or precipitated with alcohol or methyl-alcohol.

C. *Alkaline Earths*.—The hydroxyl groups of the carbohydrates combine with oxides of the alkaline earths to form more or less insoluble compounds. Levulose, for instance, forms a characteristic calcium compound. Glucose, in methyl-alcohol solution, forms a barium compound. These earthy salts are most readily precipitated out by alcohol. The sugars can be recovered from them by treatment with sulphuric, oxalic, or carbonic acids. The non-reducing di- and polysaccharides also form insoluble compounds by which they can be separated (*e.g.* cane-sugar and strontium).

D. *Benzoyl Chloride*.—Sodium hydrate is added to the urine to precipitate out the phosphates. To each litre of the clear filtrate are then added about 40 c.c. of benzoyl chloride and 400 c.c. of a 10 per cent. solution of sodium hydrate. The mixture is placed in a large stoppered-bottle, and well shaken until the smell of benzoyl chloride has disappeared. At the end of the reaction the solution must still be alkaline. The mixture is left to stand overnight on ice, and the precipitated ester filtered off, well-washed with water, and dried. It is purified by being re-crystallised, fractionally, from warm absolute alcohol. The carbohydrate is recovered by adding to each 10 grams of the ester, 7·5 grams of metallic sodium dissolved in 300 c.c. of absolute alcohol, the sodium ethylate solution being cooled to 5° C. and the finely divided ester added to it slowly, shaking well after each addition. After about 20 to 40 minutes the decomposition is complete, and a sample on being taken out and mixed with an equal quantity of water is no longer found to

give a turbidity. Sufficient sulphuric acid is now weighed out to convert the sodium into an acid sulphate, and, after mixing it with as much water as alcohol was used in the first operation, it is added to the solution. The freed benzoic acid is removed by extracting the solution with an equal volume of ether, three times. The separated ether is extracted with water, to recover traces of sugar that have dissolved in the ether, and the extract added to the alcohol sugar solution. This is then neutralised with sodium hydrate until it is only faintly acid, and finally completely neutralised with sodium carbonate. The reaction must not be alkaline. The solution is now mixed with three volumes of alcohol, and left overnight for the sodium sulphate to crystallise out. The filtrate of the feebly acid solution is evaporated *in vacuo*. The concentrated brown solution can be decolorised with lead acetate and basic lead acetate, the excess of lead being removed with sulphuretted hydrogen and purified from the latter with carbonic acid gas. This method which was at one time very extensively employed is now not often used, for it is very laborious, and benzoyl esters of other substances, which are not easily separated from the sugar compound, are also formed and carried down in the precipitate. Many researches on the reducing sugar-content of normal urines were conducted with benzoyl chloride.

E. *Hydrazones and Osazones.*—The compounds formed by many of the sugars with phenylhydrazin, and the substituted hydrazines, are more or less insoluble in water and other solvents, and so serve for their separation. From these the sugar can be recovered by appropriate treatment. (See Appendix.)

The *confirmatory tests for each variety of sugar*, &c., will now be considered.

Dextrose (glucose) is by far the most common sugar met with in the urine. Any urine that gives a marked reduction, readily ferments with yeast, and is dextro-rotatory is almost certain to contain it.

1. *Rubner's test* is a modification of the Moore-Heller test. When carried out under the following conditions a positive result is characteristic of dextrose.

Ten cubic centimetres of a concentrated solution of neutral lead acetate (one part of lead acetate to ten of distilled water) are mixed with 10 c.c. of the urine. The mixture is filtered, and ammonia carefully added to the filtrate, drop by drop, until a caseous precipitate just remains on shaking. It is then heated in a water-bath at 80° C. (not higher). If glucose is present the solution turns a beautiful red and the precipitate becomes rose or salmon-pink. If the urine is concentrated it is advisable to dilute it, so that the specific gravity does not exceed 1·010. An excess of ammonia must be avoided as it ruins the test, and the temperature must not be raised above 80° C., since lactose and maltose give a similar reaction when the solution is boiled. When the test is care-

fully carried out it is very reliable and delicate. Under the conditions described lactose gives a yellowish-pink or brown coloration, but no red precipitate ; maltose a slight yellow colour ; and levulose no colour at all.

2. *Di-phenylhydrazin.*—With this substance dextrose forms a hydrazone by which it can be distinguished from levulose, which does not form a similar insoluble compound. Galactose and the pentoses also react with di-phenylhydrazin, but their hydrazones can be distinguished from the dextrose compound by their melting-points (galactose 157° C., r-arabinose 204° to 205° C., l-arabinose 216° to 218° C., xylose 107° to 108° C.). Dextrose di-phenylhydrazone has a melting-point of 161° to 162° C.

Owing to the feeble solubility of di-phenylhydrazin in water an alcoholic solution of the requisite amount of the reagent must be used in carrying out the test. The mixture is left at the temperature of the room for two or three days, or may be heated in a water-bath for two hours. The hydrazone is precipitated out by the cautious addition of ether. It separates as small colourless prisms that are easily soluble in water and hot alcohol, but are insoluble in ether, chloroform, or benzol.

3. *Methyl-phenylhydrazin* gives a hydrazone with a melting-point of 130° C., which character distinguishes it from the similar hydrazones yielded by levulose (M.P. 158° to 160° C.) and galactose (M.P. 180° C.). It is separated out by concentrating the solution in which it forms, treating the syrup with alcohol, and recrystallising from alcohol.

4. *Benzyl-phenylhydrazin* gives the same hydrazone with both dextrose and levulose, but its melting-point (165° C.) differentiates it from the compound formed with galactose (154° C.). It appears as light yellow needles that are slightly soluble in ethyl and methyl alcohol, soluble in pyridin, and insoluble in water. The rotatory powers of its solutions are, methyl-alcohol −33°, glacial acetic acid −20·2°, pyridin −45·33°.

5. *Beta-napthyl-hydrazin* gives two hydrazones with dextrose, one that melts at 95° C. and the other at 179° C. The hydrazone of levulose melts at 152° C. They separate out as brown crystals slightly soluble in water and 95 per cent. alcohol, easily soluble in pure methyl-alcohol (+402°). The acetic acid solution is optically inactive.

6. *Para-brom-phenylhydrazin* forms a crystalline osazone with all the monosaccharides and some disaccharides. The dextrose and levulose compounds melt at 222° C., the l-arabinosazone at 196° to 208° C., the r-arabinosazone at 200° to 202° C., the l-xylose compound at 208° C., the maltosazone at 198° C. Glucuronic acid yields a crystalline hydrazone that melts at 236° C. The test may be carried out for clinical purposes as follows :—

About one-third of an inch in depth of para-brom-phenylhydrazin is introduced into the bottom of a test-tube, an equal bulk of sodium acetate is added, and the test-tube filled to one-third of its capacity with the urine. The mixture is then boiled for two minutes. Crystals, rather longer and paler than those given with phenylhydrazin, are obtained if sugar is present. Performed in this manner the test only responds to an amount of glucose that is beyond the physiological limit.

Levulose (Fructose).—When levulose occurs in a urine alone a positive fermentation test, and levo-rotation on polariscopic examination, will indicate its presence. If glucose and levulose are present together, as is generally the case, the percentage of sugar as determined by titration will be in excess of that indicated by the polariscope, and after fermentation the reducing and optical characters will be lost, provided that proteins, glucuronic acid, beta-oxybutyric acid, and other optically active substances are absent. Should the urine be levo-rotatory after complete fermentation the presence of one, or more, of these substances is indicated.

Levulose yields the same osazones with phenylhydrazin and para-brom-phenylhydrazin as levulose, but it does not form an insoluble crystalline hydrazone with di-phenylhydrazin. Its presence can be confirmed by the aid of the following tests :—

1. *Seliwanoff's Reaction.*—This test distinguishes the ketoses from the aldoses ; but since levulose (and cane-sugar) is the only ketose met with in the urine, it may be used for the detection of that sugar. The aldoses also give a reaction if the hydrochloric acid is too strong, or the heating is too prolonged. The details of the test must therefore be strictly adhered to. It is carried out as follows :—

To the urine is added an equal volume of a solution consisting of 0·5 grams of resorcin, 30 c.c. of water, and 30 c.c. of concentrated hydrochloric acid, and the mixture heated in a water-bath. If levulose is present a beautiful Burgundy-red colour develops, and a red precipitate settles out on standing. Care must be taken, however, not to confuse the rose tint given on boiling many urines with hydrochloric acid, with the typical colour reaction due to levulose. The former is only a light shade of red, and fades entirely on standing, or cooling, while the latter is a dark magenta-red, clouding the entire specimen and deepening on standing, or on rapid cooling. The colour due to levulose persists for days, and there is deposited on the bottom of the test-tube a dark red precipitate. Moreover, the red colour due to levulose appears at once, and not after prolonged heating. According to Guiart and Grimbert, the appearance of a precipitate on cooling is

more characteristic than the red colour of the solution. If the acid be neutralised with sodium carbonate, and the solution is extracted with amyl alcohol, the alcohol takes on the red colour, and on examining it with the spectroscope a band between E and B, in the green, is seen with dilute solutions, and a second band at F, in the blue, with concentrated solutions. On shaking the amyl alcohol extract repeatedly with water the colour is extracted and the alcohol appears yellow.

Borchardat describes another method of carrying out the test, which he claims gives more reliable results. A few cubic centimetres of the urine are mixed with an equal volume of 25 per cent. hydrochloric acid, a few granules of resorcin are added, and the mixture is quickly brought to the boil. A red colour should appear at once if levulose is present. The solution is then cooled, made alkaline with caustic soda solution, and extracted with acetic ether. In the presence of levulose the acetic ether extract is coloured yellow. Nitrites and indican also give the reaction if present in more than traces, and must therefore be excluded, but this modification is said not to react with urobilin and bile pigments.

2. *Pinoff's Test.*—According to Pinoff, levulose can be recognised in a mixture with other sugars, by heating 10 c.c. of the solution, with 10 c.c. of a 4 per cent. solution of ammonium molybdate, and 0·2 c.c. of glacial acetic acid, in a water-bath, at 95° to 98° C. for three minutes. Levulose gives a bright blue coloration, whereas other sugars give no colour within the time limit, but may yield a dark green colour after half an hour. Any free acid must be carefully neutralised before carrying out the test, since in the presence of free acid other sugars give a blue colour. Schoorl and Kalmthout found that with solutions containing 0·05 grams of dextrose a faint blue colour develops in ten minutes, with cane-sugar after ten minutes a green, and after twenty minutes a blue colour, and it was only with milk-sugar that twenty minutes elapsed before the green coloration was seen.

3. *Methyl-phenylhydrazin.*—This is a most important reagent for the recognition and differentiation of levulose, with which it forms an osazone consisting of bright yellow needles that melt at 158° to 160° C. A solution of the osazone in pyridin-alcohol (0·2 grams in 4·0 grams pyridin, and 6·0 grams of absolute alcohol) is dextro-rotatory (+1·40°). Dextrose and galactose do not yield osazones, but form hydrazones which melt at 130° C. and 180° C. respectively.

To obtain the osazone from the separated sugar an alcoholic solution of the reagent acidified with acetic acid (4 c.c. of a 50 per cent. solution to 10 c.c.) is mixed with a solution of the sugar and heated for five or ten minutes on the water-bath. The bright yellow osazone crystals appear in a few hours, or on the following day. They are separated off and recrystallised from 10 per cent. alcohol.

To separate dextrose and levulose when present in a mixture :—
A neutral alcoholic, and not too strongly saline, solution of the sugars

is heated with methyl-phenylhydrazin in a water-bath for some time. When a few crystals of glucose methyl-phenylhydrazone appear in the syrup it is set aside to crystallise for several days. It is then mixed with absolute alcohol and the glucose derivative separated off. The filtrate is acidified with acetic acid, heated on a water-bath for a short time, and set aside to crystallise. The resulting levulose-methyl-phenyl-osazone is purified by being recrystallised from 10 per cent. alcohol.

Methyl-phenyl-levulosazone can, according to Neuberg and Strauss, be prepared *directly from the urine by* the following procedure. The urine is acidified with a few drops of acetic acid, boiled, and filtered, to remove any albumen. It is then evaporated *in vacuo*, at 40° C., to a syrup, taking care that the reaction remains faintly acid. The syrup is mixed with half its bulk of 98 per cent. alcohol, heated on a water-bath for five minutes, cooled, and filtered. If the residue on the filter is found to have any reducing power it is mixed with a little water and extracted with alcohol once or twice more. The mixed alcoholic solutions are now, if necessary, filtered off from any flocculent precipitate that may have formed, and decolorised with animal charcoal. The sugar-content of the solution is then estimated by titration, and for each molecule of sugar found to be present, three molecules of methyl-phenylhydrazin are allowed. The sugar solution is evaporated to a small bulk (30 c.c.), cooled, and left to stand for one hour. If any precipitate forms it is filtered off. The filtrate, or original solution, is now acidified by being mixed with a weight of 50 per cent. acetic acid equal to that of the methyl-phenylhydrazin employed, and as much alcohol added as is necessary to produce a clear solution. The mixture is placed in a boiling water-bath for five minutes, or may be left at 40° C. for twenty-four hours. The osazone separates out on cooling, and adding a few drops of water, in a crystalline form if much sugar is present, but as an oil if there is only a small amount. In the latter case it is separated by strongly cooling the solution. The product is recrystallised from alcohol by cooling, or by treating it with hot water and enough pyridin to dissolve it, decolorising with animal charcoal, and filtering.

4. *Beta-napthyl-hydrazin* can also be employed to separate levulose from dextrose. With levulose it forms a hydrazone with a melting-point of 162° C., with dextrose two hydrazones that melt at 95° C. and 179° C. respectively.

The mixture of sugars is dissolved in two parts of water, and to it is added two parts of beta-napthyl-hydrazin dissolved in absolute alcohol. The mixture is left to stand for two days, shaking it at frequent intervals. The feebly soluble hydrazones of dextrose separates out first, and are filtered off. The filtrate is evaporated to dryness *in vacuo* over sulphuric acid and the residue dissolved in chloroform. It yields on recrystallisation the pure beta-napthyl-phenylhydrazone of levulose.

5. *Benzyl - phenylhydrazin* forms hydrazones with levulose,

dextrose, and other sugars. The compounds formed with levulose and dextrose have the same melting-point (165° to 170° C.), but the latter is decomposed into its constituents by boiling water, whereas the former is not affected. L-arabinose yields a hydrazone with a melting-point of 170° to 174° C., but it is insoluble in alcohol. The hydrazone of r-arabinose melts at 185° C. Galactose forms a hydrazone with a melting-point of 154° to 158° C. that is only feebly soluble in alcohol, but appears much later than the arabinose compounds.

To prepare the hydrazones a solution of the sugar in 96 per cent. alcohol is mixed with the calculated amount of benzyl-phenylhydrazin, and heated in a water-bath for five or six hours. The solution is evaporated down, and the product recrystallised out of alcohol. The hydrazones of the aldoses and ketoses may then be distinguished by their behaviour with boiling water.

Lactose (Milk-sugar).—Lactose reduces alkaline solutions of copper and bismuth, although more slowly than dextrose, but does not reduce Barfoed's solution. Boiling with dilute mineral acids increases the reducing power of its solutions, but boiling with citric acid produces no change. Lactose does not ferment with ordinary brewer's yeast within twenty-four hours, but it may be slowly broken down by contaminating bacteria. This spurious fermentation may be prevented by the addition of sodium, or ammonium, fluoride. A urine which still reduces, and is dextrorotatory, after being fermented with yeast probably contains lactose, or possibly a pentose. The confirmatory tests are as follows :—

1. *Rubner's Test.*—On carrying out this test as described for dextrose, a yellowish-pink or brown colour is obtained, but the precipitate is white. If the solution is boiled it turns yellow, then intense brick-red. On standing the fluid becomes colourless, and a copper-red precipitate settles out. Maltose gives a similar reaction.

2. *Wöhlk (Malfatti) Test.*—Lactose may be detected in the urine by mixing it with half its volume of concentrated ammonia, and heating the mixture in a water-bath that is not quite boiling for from five to fifteen minutes. The mixture turns red if lactose is present.

In Malfatti's modification of this test 5 c.c. of the urine are mixed with 2 to 5 c.c. of strong ammonia, and five drops of caustic potash solution added. The mixture is heated, but not quite to boiling, in a water-bath. In the presence of milk-sugar a red coloration develops in about five minutes. By this test it is stated that 0·1 per cent. of lactose can be detected in an otherwise sugar-free urine. Maltose gives the same reaction, but with glucose a yellow or brown colour is obtained.

3. *Mucic Acid Test.*—Mucic acid is a characteristic derivative of galactose. It is formed, along with saccharic acid, when lactose is hydrolised and oxidised, and owing to its insolubility, the acid may be readily prepared and separated from solutions containing lactose. Although the reaction is satisfactory for pure solutions, only some 50 to 60 per cent. of the theoretical yield is often obtained from the urine, so that small amounts may be easily missed.

According to Langstein and Steinitz, the test can be carried out as follows. The urine is treated with lead acetate and ammonia, and the resulting precipitate filtered off. This is washed with water, and decomposed with sulphuretted hydrogen. The lead sulphide is removed by filtration and the excess of sulphuretted hydrogen expelled from the filtrate by warming. It is then evaporated down three times with ammonia (sp. gr. 1·2). The presence of mucic acid is shown by dissolving in ammonia, evaporating, and subjecting the residue to dry distillation, when pyrrol is formed. This is recognised by the red violet coloration it gives with a pinewood splinter moistened with hydrochloric acid.

Bauer's method is as follows. 100 c.c. of the urine are mixed with 20 c.c. of pure concentrated nitric acid (sp. gr. 1·4), in a small beaker, and placed in a boiling water-bath. At first the solution is dark-coloured, but later becomes clear yellow. When this stage is reached, and it is seen to contain a fine white precipitate, generally when it has evaporated down to about 20 c.c., the beaker is removed from the bath and its contents poured into a smaller, into which any precipitate is washed with two small portions of water. It is then left to cool over night. After being diluted with water the precipitate is filtered off, repeatedly washed with cold distilled water, and dried. Mucic acid has a melting-point of 213° to 215° C., and after recrystallising from boiling water 217° to 225° C. The precipitate may also be dissolved in ammonia and tested for pyrrol, as in the preceding method.

4. *Phenylhydrazin.*—The phenylosazone of lactose differs in its appearance, melting-point (about 210° to 212° C.), and optical activities (pyridin-alcohol solution ± 0·00), from those of other sugars, but owing to its being relatively soluble in water, and the small amount of lactose generally present (under 1 per cent.), the osazone cannot usually be prepared directly from the urine. A negative phenylhydrazin test with a reducing urine is therefore suggestive of the presence of lactose. To prepare the osazone the sugar must be first isolated from the urine, or the urine may be treated with Patein-Dufau's reagent, which precipitates out uric acid, creatinin, albumen, &c., and yields a colourless filtrate containing the sugar.

Patein-Dufau Reagent.—220 grams of red oxide of mercury are

mixed with 160 c.c. of nitric acid (sp. gr. 1·39) in a porcelain basin. After standing for five or six minutes the mixture is diluted with 160 c.c. of water, and heated until the oxide is completely dissolved. On cooling, 40 c.c. of a 10 per cent. solution of sodium hydroxide are gradually added, with constant stirring, and the mixture diluted to 1000 c.c. The solution is filtered, and preserved in dark glass bottles. One part of the reagent is used to precipitate four parts of urine, and the filtrate used for the phenylhydrazin test, after removing the excess of mercury with sulphuretted hydrogen.

If a urine suspected to contain lactose is boiled with 5 per cent. sulphuric acid for a short time, and the excess of acid neutralised with ammonia, the phenylhydrazin test should show crystals of dextrosazone, and, with proper precautions, galactosazone also.

5. *Beta-benzyl-phenylhydrazin.*—With this reagent lactose forms a hydrazone that melts at 128° C. It appears as light yellow needles, slightly soluble in alcohol, and soluble in methyl-alcohol. Its methyl-alcohol solution is levo-rotatory (−25·7°).

Pentoses.—A urine containing a pentose gives a reaction with an alkaline solution of copper. When dextrose is not also present the reduction is stated not to take place at once, but only after heating for some time, and then occur suddenly throughout the whole bulk of the fluid. Nylander's solution is only slightly reduced, a grey precipitate being formed. A pentose-containing urine does not ferment with yeast. It may be either dextro-rotatory, or inactive, according to the variety that is present. Such a urine also gives the phloroglucin test. From the results of the reduction and fermentation tests a pentose may be easily mistaken for lactose, but a pentose-containing urine should show a characteristic spectrum on examining an amyl alcohol extract of the phloroglucin test with the spectroscope.

As glucuronic acid gives similar results, confirmatory tests must, however, be applied. These are as follows :—

1. *Orcin Tests.*—The most easily applied of the confirmatory tests for the presence of a pentose is the orcin reaction. When a solution of a pentose is heated with strong hydrochloric acid and orcin the fluid develops a violet-blue colour, or a green if iron is present. To be reliable, however, the test must be very carefully carried out in every detail, and the reagents employed must be of the exact strength and kind described.[1] Several modifications of the test have been described, but Bial's is the one most commonly employed clinically.

[1] The hydrochloric acid must be pure, and sp. gr. 1·195, the commercial acid will not do : orcin is not the same thing as the dye orcein, with which I have more than once seen the test attempted.

(a) *Salkowski's Method.*—A few cubic centimetres of the urine are mixed with an equal quantity of strong hydrochloric acid (sp. gr. 1·195), and a few granules of orcin, in a test-tube. The mixture is then heated in the flame for twenty to thirty seconds. If a pentose is present the solution turns a reddish-blue, or, if the acid contains traces of iron, a dark green colour, and a dark blue, or green, precipitate forms. If the solution is cooled until it is just warm, and extracted with amyl alcohol, it gives a beautiful dark blue, or green, extract, which on examination with the spectroscope shows a characteristic absorption band between C and D (red and yellow). Glucuronic acid gives the same colour reactions and spectrum as the pentoses, but as a rule it is not split off from its combinations by such brief heating.

(b) *Bial's Modification.*—Four or five cubic centimetres of a reagent made by mixing 500 c.c. of fuming hydrochloric acid (sp. gr. 1·195), 1 gram of orcin, and twenty-five drops of a 10 per cent. solution of perchloride of iron, are heated to boiling, and then removed from the flame. The urine is immediately added drop by drop, agitating the liquid, and observing its colour between each addition, until either the characteristic result is obtained, or 1 c.c. of the urine has been added in all. If a pentose is present a green colour should appear at once, or almost immediately, and an amyl alcohol extract of the cooled fluid should yield a green fluid, which shows the same spectrum as is obtained with Salkowski's modification. It is claimed by Bial that his test is much more sensitive than the original method, and yet does not give a reaction with glucuronic acid when properly performed.

(c) *Jolles' Test.*—Jolles does not consider that Bial's reaction can be relied upon to differentiate the pentoses from glucuronic acid, and recommends the following procedure:—10 to 20 c.c. of the urine are mixed with 1 gram of phenylhydrazin hydrochloride, and 2 grams of sodium acetate. The mixture is shaken, and heated for about an hour in a boiling water-bath. It is then cooled for a couple of hours in water. The resulting osazone is filtered off on to an asbestos filter, washed with 3 or 4 c.c. of cold water, and, with the asbestos, introduced into a distillation flask, containing 20 c.c. of water and 5 c.c. of concentrated hydrochloric acid. Five c.c. are distilled over into 5 c.c. of cold water, and 1 c.c. of the mixture tested with Bial's reagent. If a pentose is present an intense green colour develops, and the characteristic spectrum is seen. Jolles states that by this method 0·05 per cent. of arabinose can be detected, but that glucose and glucuronic acid give no reaction.

(d) *Neumann's Test.*—Ten drops of the suspected urine are mixed in a test-tube with 5 c.c. of glacial acetic acid (99 per cent.) and a drop of a 5 per cent. alcoholic solution of orcin. The mixture is shaken, and raised quite to the boiling-point. The test-tube is then held in a test-tube holder, and concentrated sulphuric acid dropped in, with constant shaking, until a faint violet-blue colour appears. As a rule not more than fifty drops of sulphuric acid are necessary, and an excess obscures the tint.

2. *Phenylhydrazin.*—According to Salkowski, this test is best performed as follows :—

200 c.c. of the urine are placed in a beaker, and mixed with 5 grams of phenylhydrazin and the same quantity of 50 per cent. acetic acid. The mixture is well shaken, gently heated on a wire gauze, and then in a water-bath, but not to boiling. The fluid is filtered while hot, and cooled by placing the beaker in cold water. The resulting pentosazone crystals are filtered off, and purified by repeated recrystallisation from hot water. They differ from glucosazone crystals in their appearance microscopically, their greater solubility in hot water, and their much lower melting-point. The last varies from 155° to 168° C. according to the way in which the heat is applied, and the variety of pentose present, the inactive forms giving an osazone with a higher melting-point than the active varieties.

Should the *urine contain both pentose and hexoses*, Külz and Vogel suggest that they can be separated by the following procedure :—

From 1·6 to 3·2 litres of the urines are taken, and, for each 100 grams of dextrose, 200 grams of phenylhydrazin and 100 grams of glacial acetic acid are added. The mixture is heated on a water-bath for an hour and a half, cooled, and filtered. The filtrate is again heated on the water-bath for an hour and a half, and filtered. The combined precipitates are well washed with cold water, and the pentosazone extracted by digesting with water at 60° C., one litre of water for each 100 grams of sugar being used, and the digestion being continued for twelve hours. This is repeated fifteen times. The hot extracts are filtered, and allowed to cool. The pentosazone will then separate out. It is purified by recrystallisation from water at 60° C., or from acetone, till the melting-point is constant.

The variety of pentose present can be determined from a consideration of the following characters :—

l-arabinose.—1. The urine, or solution of the sugar, is dextro-rotatory (+104·4°).

2. The phenylosazone forms a voluminous precipitate, consisting of yellow crystals, which are insoluble in cold water, ether, benzol, and ligroin, and are soluble in hot water, alcohol, acetone, and pyridin. A 4 per cent. alcoholic solution, when freshly prepared, is dextro-rotatory (+18·9°), but on standing becomes optically inactive. The pyridin-alcohol solution is dextro-rotatory (+1·1°). The crystals washed with water and recrystallised from hot water and acetone melt at 160° C. on rapid heating.

3. *Di-phenylhydrazin.*—The hydrazone that l-arabinose forms with di-phenylhydrazin is one of its most insoluble compounds, and is therefore of great use in separating and identifying it. It appears

as white needles, which on being rapidly heated melt at 216° to
218° C. Its pyridin-alcohol solution is slightly dextro-rotatory
(+ 0·42°). It can be prepared directly from the urine in the fol-
lowing manner (Neuberg and Wohlgemuth) :—

100 c.c. of the urine are feebly acidified with acetic acid, evaporated
to 40 c.c., and mixed with an equal volume of alcohol. The precipitate
that forms after standing for two hours is filtered off, and washed with
50 per cent. alcohol. The filtrate is mixed with 1·4 grams of di-phenylhy-
drazin, and heated on a water-bath for half an hour, the loss of alcohol
from evaporation being made up as required. It is then left to cool,
when the crystalline hydrazone will separate out. On treating each
gram of the hydrazone with 4 c.c. of formalin and a little water it is
broken up into formaldehyde-diphenylhydrazin and the pentose. The
former can be separated by shaking out with ether, leaving the sugar
in solution, from which it can be crystallised.

4. *Para-brom-phenylhydrazin.*—With para-brom-phenylhydrazin
l-arabinose forms a very characteristic insoluble hydrazone, by
which it can be distinguished from xylose and the hexoses. Its
melting-point of 160° to 162° C. also serves to distinguish this
sugar from glucuronic acid, the para-brom-phenylhydrazin com-
pound of which melts at 236° C.

The hydrazone is prepared by mixing each part of sugar with a freshly
prepared solution, consisting of one part of para-brom-phenylhydrazin,
three and a half parts of 50 per cent. acetic acid, and twelve parts of
water, and standing for some time, when it separates out as fine crystals.

l-arabinose also forms an osazone with para-brom-phenylhy-
drazin, which is easily soluble in hot water, alcohol, acetone, benzol,
ether, and pyridin, but is feebly soluble in cold water, and insoluble
in ligroin. Its pyridin-alcohol solution is feebly dextro-rotatory
(+ 0·28°). From alcohol it crystallises as yellow needles, and from
pyridin as six-sided plates. It softens at 185° C., and melts at
196° to 200° C.

5. *Methyl-phenylhydrazin* forms a hydrazone that is easily
soluble in alcohol and pyridin, slightly soluble in water, and in-
soluble in ether. Its alcoholic solution is dextro-rotatory (+ 4·3°).
A solution in acetic acid is levo-rotatory (– 21·8°). A pyridin solu-
tion is optically inactive. It forms yellow crystals that melt at
161° to 164° C.

6. *Benzyl-phenylhydrazin* forms an osazone consisting of white
crystals that are soluble in methyl alcohol (– 12·1°), and glacial acetic
acid (– 14·6°). It has a melting-point of 170° to 174° C.

i-arabinose.—1. The urine is optically inactive.

2. *Phenylhydrazin* forms an osazone, consisting of yellow needles or prisms, which when pure melt at 166° to 168° C. The osazone prepared directly from the urine usually melts at a much lower temperature, generally at about 156° C.

3. *Di-phenylhydrazin.*—On warming an alcoholic solution of the sugar with an equivalent mass of di-phenylhydrazin, the hydrazone separates out as long white needles, that are insoluble in cold water and alcohol, slightly soluble in chloroform, hot water, and alcohol, and readily soluble in acetic acid and pyridin. The pure product melts at 204° to 205° C.

4. *Para-brom-phenylhydrazin* gives a hydrazone that is easily soluble in pyridin, but less soluble in water, alcohol, and ether. It has a melting-point of 160° C. Para-brom-phenylhydrazin also forms an osazone, consisting of long yellow needles that melt at 200° to 202° C.

5. *Methyl-phenylhydrazin* forms a hydrazone that is easily soluble in water, pyridin, and hot alcohol, less soluble in cold alcohol, acetone, and chloroform, and is insoluble in benzol. Crystallised out from alcohol it melts at 173° C.

6. *Benzyl-phenylhydrazin* forms a hydrazone, consisting of light yellow needles, that are soluble in hot water, alcohol, and chloroform, are less soluble in ether, benzol, and ligroin, and are easily soluble in pyridin. It melts at 185° C.

l-xylose.—1. Its solution is dextro-rotatory (+18·10°).

2. *Phenylhydrazin* gives an osazone that crystallises out in light yellow shining needles, or plates. It is easily soluble in ether and acetone, feebly soluble in water, and easily soluble in alcohol, but less so in acetone. A solution in alcohol is strongly levo-rotatory (– 43·4°). The melting-points given by different authors vary between 152° C. and 170° C. According to Wheeler and Tollens, the pure product melts at 159° to 160° C.

3. *Di-phenylhydrazin* yields a hydrazone with a melting-point of 107° to 108° C. It is, however, much more soluble than the corresponding arabinose compound, and so serves to separate that sugar from xylose.

4. *Para-brom-phenylhydrazin* gives only a soluble hydrazone with xylose, and so can be differentiated from arabinose. It forms an insoluble osazone, consisting of yellow needles, solutions of which have the same rotatory powers as those of the arabinose compound. Its melting-point is also very similar (208° C.).

5. *Methyl-phenylhydrazin* gives a soluble hydrazone, consisting of yellow crystals that dissolve in water, alcohol, acetone, acetic, ether, chloroform, and pyridin. It has a melting-point of 108° to 110° C.

6. *Benzyl-phenylhydrazin.*—The hydrazone of xylose forms needles with a melting-point of 93° C. It is only feebly soluble in water, is more easily soluble in ether, and is very soluble in alcohol. Its alcoholic solution is strongly levo-rotatory (– 33°).

7. *Brucin.*—With brucin xylose forms a crystalline salt, on being warmed with a faintly alkaline solution. It separates as rhombic tables, and has a melting-point of 172° to 174° C. It is almost insoluble in cold water and alcohol.

8. *Rubner's Test.*—On applying Rubner's test and boiling, xylose gives a deep orange precipitate.

9. *Xylonic Acid Test.*—The most important and characteristic evidence of the presence of xylose in a solution is obtained by converting it into xylonic acid. This is effected by oxidising it with bromine, and separating out the acid as the insoluble double cadmium-bromine salt. The test is carried out as follows :—

0·2 gram of xylose, or double the volume of the solution, 1 cm. of water, 0·25 gram (seven to eight drops) of bromine, and 0·5 gram of cadmium carbonate are mixed in a test-tube, shaken, and gently warmed. The loosely corked test-tube is then set aside, for twelve to twenty-four hours. The contents are now evaporated to dryness in a porcelain basin, and the residue dissolved in 4 to 5 c.c. of water. The solution is filtered, evaporated to dryness, and mixed with 1 c.c. of alcohol. If pure xylose was present a crystalline precipitate separates out, and on microscopical examination this is seen to consist of needle-like, or whetstone-like, crystals.

Glucuronic Acid.—As a rule the glucuronic acid compounds met with in the urine only reduce alkaline solutions of copper after prolonged boiling, but if the urine has been previously heated with 1 per cent. sulphuric acid for from one to five minutes, an immediate reduction occurs. Urocholic acid and paramidophenyl-glucuronic acid reduce Fehling's solution as readily as dextrose without any preliminary treatment, and phenol-glucuronic acid reduces after being boiled with an alkaline solution of copper for a short time. Like the pentoses, glucuronic acid and compound glucuronates are not fermented by yeast. Although paired glucuronic acid does not give the phloroglucin and orcin tests until the compounds have been decomposed and the glucuronic acid set free by prolonged heating, or boiling with dilute mineral acids, some are more readily decomposed than others, and may give rise to difficulties with these tests unless they are carefully carried out.

1. A urine containing compound glucuronates is levo-rotatory, but, since the free acid, and its alkaline salts, are dextro-rotatory, on boiling with dilute acid the optical activity will be changed. If the urine contains dextrose, its dextro-rotatory power will be raised. The levo-rotation of normal urines (about 0·05 per cent.) may be increased to 0·25 per cent. by the presence of indoxyl and phenol-glucuronic acids, but if it is over 0·15 per cent. it is probable that they are present in excess. The presence of albumen, and other levo-rotatory substances, must first be excluded, although albumen up to 0·5 per cent. may be neglected, as this amount does not appreciably affect the optical activity of the urine. If a reaction for acetone is given, the levo-rotation may be due to the presence of beta-oxybutyric acid. This should be removed by extracting the urine three times with ether before taking the reading. If the urine is dark-coloured, and it is necessary to clear it for examination with the polariscope, it should be remembered that some glucuronates (e.g. uroculoric acid, phenol-, menthol-, and napthol-glucuronic acid) are precipitated by lead acetate, while others, such as the camphor compound, are not. The urine must be acid in reaction, since the levo-rotation is less in alkaline solutions. An optically inactive, or even a dextro-rotatory, urine may contain glucuronates, for glucuronic acid is set free spontaneously from some, such as the menthol compound.

2. *Phenylhydrazin.*—Most paired glucuronates do not form a crystalline compound with phenylhydrazin when the test is applied directly to the urine, but after boiling with dilute sulphuric or hydrochloric acid, or even in some instances on simply heating for some time with water, the glucuronic acid is set free, and yields crystals that may be easily mistaken for the osazone of a sugar. The phenylhydrazin compound of glucuronic acid is readily soluble in hot alcohol, but generally separates from this solution, after diluting with water and boiling, in an amorphous form. The crystals are feebly soluble in water and hot benzol, are easily soluble in acetone, and very easily dissolve in pyridin, yielding a levo-rotatory solution. In methyl-alcohol they dissolve with ease, being thus distinguished from dextrosazone, which is only very slightly soluble. The phenylhydrazin compound of glucuronic acid dissolves in about one to two minutes when irrigated under the microscope with 33 per cent. sulphuric acid.

Examined under the microscope with polarised light they are invisible, unlike the osazones of the sugar, which appear bright, and of a green and red colour. The melting-point of the crystalline variety is 114° to 115° C., but the amorphous form shows no change

until the temperature has been raised to 150° C. or so. With specimens isolated from the urine the melting-point may be anywhere between 114° and 217° C.

Since the formula of the phenylhydrazin compound of glycuronic is $C_{42}H_{48}N_{10}O_{10}$, it is calculated to yield 16·4 per cent. of nitrogen on combustion. It is not at all an easy matter, however, to obtain a sufficiently pure specimen from the urine to allow of an accurate determination of either the melting-point or the nitrogen content.

As a rule the glucuronates cannot be satisfactorily differentiated from traces of sugar by the phenylhydrazin test, since they do not yield a pure product in an amount sufficient for a complete examination.

3. *Para-brom-phenylhydrazin* gives the most characteristic crystalline compound by which glucuronic acid can be recognised. Unlike the corresponding compounds yielded by the sugars, it is insoluble in absolute alcohol. The raw product melts at 200° to 206° C., but after being recrystallised from hot 60 per cent. alcohol it has a melting-point of 236° C. Its solution in pyridin-alcohol is strongly levo-rotatory (− 7·25°). The test is carried out as follows :—

A boiling solution of 5 grams of para-brom-phenylhydrazin, and 6 grams of sodium acetate, is added to the urine, in which the glucuronic acid has been previously set free, or a solution containing the separated acid, and heated on the water-bath to 60° C. At first the mixture is clear, but in from five to ten minutes a yellow precipitate separates out. The solution is now allowed to cool, the crystals are filtered off, and the filtrate is heated afresh. A second crop of crystals separates out. These are filtered off, and the filtrate is again heated on the water-bath, the process being repeated so long as a precipitate forms. The crystals on the filter are carefully washed with a little warm water, then with absolute alcohol, dried, and the melting-point is taken.

4. *Naphtho-resorcinol Test* (*Tollens*).—On being heated with naphtho-resorcinol and hydrochloric acid, glucuronic acid forms a blue substance, soluble in ether. The pentoses do not give this reaction, so that glucuronic acid can be detected by means of it in their presence. The test is carried out as follows :—

Five or six cubic centimetres of the urine, or a piece of the solid glucuronic the size of a pea dissolved in 5 to 6 c.c. of water, are mixed with 0·5 to 1·0 c.c. of a 1 per cent. alcoholic solution of naphtho-resorcinol, and 5 to 7 c.c. of hydrochloric acid (1·19), and gently boiled in a wide test-tube for one minute. After standing for four minutes the liquid is cooled, mixed with an equal volume of ether, and well shaken. If glucuronic acid is present, the ethereal solution has a blue or red colour, and exhibits a blue fluorescence. Examined with the spectroscope, it shows a band slightly to the right of the D-line. As a reaction is obtained

with 0·1 per cent., or less, a positive result is given by many normal urines. The presence of indoxyl may vitiate the test, and it should therefore be previously removed by treating the urine with mercuric acetate.

5. *Quinine* forms with glucuronic acid an insoluble salt, consisting of microscopic needles, with a melting-point of 204° C., which are strongly dextro-rotatory (+138·6°). The solution of glucuronic acid is heated to boiling, and quinine added until it no longer dissolves. On cooling the quinine salt separates out.

6. *Brucine* also forms an insoluble salt, with a melting-point of 200° C.

7. *Benzoyl chloride.*—On shaking a solution of free glucuronic acid with benzoyl chloride and sodium hydrate (in 10 per cent. solution), it is precipitated out as dibenzoyl-glucuronic acid. The precipitate is insoluble in water, but is easily soluble in alcohol, particularly in warm alcohol. It reduces Fehling's solution, and melts at 107° C. If too much soda is used the precipitation is interfered with, so that for each molecule of glucuronic acid as nearly as possible 9 molecules of benzoyl chloride, and 12 of sodium hydrate, should be employed.

Separation (a) *by Lead.*—Paired glucuronic acid may be separated from the urine by concentrating, treating with lead acetate, then with tribasic lead acetate, and eventually with ammonia and tribasic lead acetate. The lead precipitate is washed and suspended in water, treated with sulphuretted hydrogen, the lead sulphate removed by filtration, and the filtrate heated at 100° C. with 1 per cent. sulphuric acid, in a flask provided with reflux condenser. The fluid is now neutralised with sodium carbonate, and treated with para-brom-phenylhydrazin acetate. After heating for about ten minutes the para-brom-phenylhydrazin separates out.

(b) *Barium.*—Glucuronic acid may also be separated as the insoluble barium salt. The urine is decolorised with animal charcoal, and evaporated to a syrup. It is then digested with a large quantity of damp barium hydrate, at a gentle heat, on a water-bath. The mixture is extracted with absolute alcohol, and the residue mixed with water, and filtered. More baryta is added to the filtrate, and it is again filtered, and the filtrate evaporated down on a water-bath. An amorphous barium compound of glucuronic acid separates out. This is washed with water, decomposed with sulphuric acid, the barium sulphate filtered off, and the filtrate evaporated down, and dried *in vacuo.* Crystals of the anhydride of glucuronic acid can thus be obtained.

Maltose.—Maltose reduces alkaline solutions of copper and bismuth, but not Barfoed's reagent until after the mixture has been heated for some time. It is fermented by yeast as easily as dextrose, and without previous inversion by acids. Its solutions are

strongly dextro-rotatory, deflecting the plane of polarised light more than twice as much to the right as a solution of dextrose of equal strength. Since the amount of cuprous oxide precipitated by maltose from Fehling's solution is only 62 per cent. of that produced by an equal weight of dextrose, the readings obtained with the polariscope, and by reduction, differ very widely when a urine containing maltose is examined by these two methods. After hydrolysis with dilute acid the urine becomes less dextro-rotatory, but reduces Fehling's solution to a greater extent than before.

1. *Phenylhydrazin.*—Maltose is most surely recognised and differentiated by the osazone that it forms with phenylhydrazin. This is prepared by prolonged heating ($1\frac{1}{2}$ hours on the water-bath), and does not separate out from the hot solution, but only on cooling. It appears as fine yellow needles, in marked contrast to the coarse crystals of dextrosazone and levulosazone. On taking the melting-point of the purified product it is found to soften at 190° to 193° C., and to melt, on rapid heating, at 202° to 208° C. Its solution in pyridin-alcohol is dextro-rotatory ($+1\cdot30°$), in contrast to dextrosazone and levulosazone, which are levo-rotatory ($-1\cdot30°$). Maltosazone is much more easily soluble in hot water than dextrosazone, and so can be separated by fractional crystallisation. It is also more easily soluble in acetone, and can be further purified by extraction with 50 per cent. acetone.

2. *Para-brom-phenylhydrazin.*—Maltose does not give an insoluble crystalline hydrazone with para-brom-phenylhydrazin, but it forms an osazone with a melting-point of 198° C. The osazone is prepared by standing an alcoholic solution of the sugar with para-brom-phenylhydrazin for several days at 40° C. It appears as needles that are soluble in hot alcohol and acetone, less soluble in acetic, ether, benzol, and chloroform, and are insoluble in ether and ligroin.

Isomaltose.—Isomaltose is dextro-rotatory, and gives much the same reactions as maltose. It reduces Nylander's and Fehling's solutions to four-ninths the extent of dextrose, but only ferments with yeast after prolonged treatment, and gives a different osazone with phenylhydrazin. It is by the characters of its osazone that it has generally been recognised in the urine. It has also been separated by fermenting the carbohydrates precipitated out with benzoyl chloride.

Phenylhydrazin.—On heating a 20 per cent. solution of isomaltose with phenylhydrazin acetate, and adding two volumes of cold water, the osazone separates as a flocculent yellow precipitate. On microscopical

examination this is found to consist of spherical aggregates of bent yellow needles, that are more readily soluble in hot water, and hot alcohol, than maltosazone, but are insoluble in ether, acetone, and water-free acetic acid. On drying they turn orange-yellow, and at 100° C. dark yellow. At 142° C. they soften, and melt at 145° (Ost) or 153° (Fischer). They can be purified by recrystallisation from warm acetic acid. Their acetic ether solution is levo-rotatory ($-20°$).

From a mixture of osazones prepared from the urine isomalto-sazone can be separated out, along with maltosazone, by its solubility in hot water. From maltosazone it can be differentiated by its comparative insolubility in acetone. Mayer points out, however, that it is most unsatisfactory to depend solely on the melting-point of an osazone for its recognition, as several observers have done in the case of isomaltose (*e.g.* Pavy and Siau) ; and he suggests that, in some instances at least, the sugar regarded as isomaltose was probably glucuronic acid.

Galactose.—Galactose gives the ordinary reduction tests, like other monosaccharides. It is not fermented by brewer's yeast, but is slowly broken down by bacteria. A urine which contains only galactose shows no gas formation in six hours. It is more strongly dextro-rotatory than either dextrose or lactose ($[a]_b$ for dextrose $+52·5°$, for lactose $+52·5°$, for galactose $+81°$). It may be distinguished from other sugars by the following tests :—

1. *Phenylhydrazin.*—With phenylhydrazin galactose forms an osazone which is distinguished from dextrosazone by its melting-point, and from lactosazone by its being less soluble in both cold and hot water. The osazone separates out in stout yellow needles, that are only slightly soluble in cold water, more soluble in hot water and alcohol, and easily soluble in hot 60 per cent. alcohol. In pyridin-alcohol the rotation is $+0·48°$ (Neuberg). The melting-point varies very much with the purity of the product, the un-purified osazone melting at 171° to 174° C., the purified crystals at 194° to 195° C. The osazone can be prepared direct in the usual way from urines rich in galactose ; but when only small quantities are present the urine must be previously treated with the Patein-Dufau reagent, or the sugar must be isolated. Any admixed lacto-sazone can be removed by washing the crystals with hot water, and the galactosazone be purified by recrystallising from dilute alcohol and washing with ether, in which it is insoluble.

2. *Methyl-phenylhydrazin.*—The methyl-phenylhydrazone is the most characteristic compound by which galactose can be recognised and separated, in the presence of other sugars. It forms colourless needles, with a melting-point of 180° to 188° C., that are

only slightly soluble in water and alcohol, but are easily soluble in methyl-alcohol. The hydrazone is prepared by treating a hot concentrated solution of the sugar with the calculated amount of methyl-phenylhydrazin.

3. *Di-phenylhydrazin.*—With di-phenylhydrazin galactose forms an hydrazone with a melting-point of 157° C., which cannot, however, be distinguished in practice from the hydrazone formed with dextrose (M.P. 161° C.).

4. *Benzyl-phenylhydrazin.*—Galactose forms a hydrazone consisting of light yellow needles, slightly soluble in water and alcohol, that melts at 154° to 158° C. Its solution in pyridin is levo-rotatory ($-14.63°$), and the methyl-alcohol solution is also levo-rotatory ($-17.2°$).

5. *Mucic Acid.*—On oxidising galactose with nitric acid a feebly soluble, sandy, crystalline powder, consisting of mucic acid, is formed. The same procedure may be followed as for the preparation of mucic acid from lactose, or the sugar may be isolated and treated in the following manner :—

The sugar is mixed with about twelve times its bulk of nitric acid (sp. gr. 1·15), and heated for some time on a water-bath. The excess of nitric acid is then evaporated off, and the residue, mixed with a little water, is left to crystallise out until the following day. The precipitate that forms is washed with water, and the crystalline mucic acid removed by filtration. This is purified by further washing. On microscopical examination it is found to consist of short prisms, which are insoluble in alcohol and ether, and have a melting-point of 225° C. on being quickly heated. On dissolving the mucic acid in ammonia, evaporating, and subjecting the product to dry distillation, CO_2, H_2O, NH_2, and pyrrol are formed. The latter can be recognised by the red-violet colour given by a pine-splinter moistened with hydrochloric acid. Mucic acid also gives a characteristic yellow to reddish-yellow coloration with a reagent consisting of two drops of perchloride of iron, two drops of strong hydrochloric acid, and 100 c.c. of water.

6. *Galactose pentabenzoate* crystallises in microscopic needles which melt at 165° C. It is, however, mixed with yellowish drops of an amorphous modification which melts at 82° C.

Laiose.—This substance has not been obtained in a crystalline form, so that the reactions of the pure product are not definitely known. Its solutions are levo-rotatory ($-26.07°$). It is not fermented by yeast, but reduces Fehling's solution, although to a less extent than dextrose or levulose, and only after prolonged boiling. It gives a slight reaction with Moore's test, and forms with phenylhydrazin an oily compound. It has been variously regarded as a hexose (levulose), a pentose (d-xylose), and a heptose.

To separate laiose from the urine it is treated with lead acetate, and the resulting precipitate filtered off. Ammonia is then added to the filtrate. This second precipitate, which contains the laiose and any other sugars, is suspended in water, and decomposed with a stream of sulphuretted hydrogen. The filtrate is evaporated *in vacuo*, over sulphuric acid, to a syrup, and the syrup treated with methyl-alcohol. The sugar is then precipitated out with a methyl-alcohol solution of baryta, and quickly filtered off. The filtrate is left to stand over sulphuric acid, treated with carbonic acid, and the filtrate from this concentrated *in vacuo*, to remove the methyl-alcohol. The residue is dissolved in water, and the baryta still in solution is precipitated with sulphuric acid, and the chlorides removed as a silver salt.

Cane-sugar.—Cane-sugar is introduced into the urine by malingerers, or may accidentally find its way there. Pure cane-sugar has no reducing action on cupric oxide, but, since the commercial variety contains other sugars as impurities, it may give a positive, although not quite typical, reaction with Trommer's test or Fehling's solution. For the same reason phenylhydrazin may also give a few osazone crystals. A urine containing cane-sugar ferments only very slowly, is often of a high specific gravity, and is dextro-rotatory. On boiling with dilute hydrochloric acid for twenty to forty minutes, and neutralising with sodium carbonate, it will be found to be levo-rotatory, from inversion of the cane-sugar. It will then also give the typical tests for dextrose and levulose.

Other Reducing Substances.—In addition to those already described, other reducing substances have been reported as present in the urine by several observers.

Salkowski and Blumenthal separated from the urine of several cases of pneumonia a fermentable, dextro-rotatory body, yielding with phenylhydrazin an osazone having a melting-point of 195° C. and a nitrogen content of 16·06 per cent.

Jacoby described a reducing substance, recovered from the urine of a case of Addison's disease, that yielded an osazone with a melting-point of 175° to 180° C.

Rosenberg isolated a sugar, which he regarded as a heptose, from the urine of a case of diabetes. It reduced alkaline solutions of copper, and formed with phenylhydrazin an osazone that melted at 195° C. The osazone was soluble in pyridin, and this solution was optically inactive.

Geelmuyden gave the name " paidose " to a sugar that he isolated from the urine of diabetic children. It was optically inactive, slowly reduced Fehling's solution, and gave an osazone

with a melting-point of 175° to 190° C. It did not give the phloro-glucin and orcin reactions.

Glucosamine.—An amino sugar, glucosamine ($C_6H_{11}O_5.NH_2$), prepared from chitinin, has been found in the urine after it has been given by the mouth, or subcutaneously. It reduces alkaline solutions of copper, but not as strongly as dextrose, and can be differentiated from glucose by converting it into the tetrabenzoate. According to Kueny, the melting-point of this compound is 197° to 198° C., according to Pum, 203° C. On decomposing the benzoate the glucosamine can be recovered and identified.

Animal Gum (Landwehr) is probably not one, but a group of bodies, precipitated from the urine by alcohol. It is said to be present in traces in all urines, and to be increased in some pathological conditions. It is slightly dextro-rotatory, and is not fermented by yeast. With the copper tests it gives a precipitate which does not blacken on boiling, but, after prolonged heating with dilute sulphuric acid, it yields a reducing substance. Unlike glycogen, it does not give a colour reaction with iodine.

Glycogen (or **Erythrodextrin**).—Urines containing this substance are dextro-rotatory. They do not reduce alkaline solutions of copper at once, but on prolonged heating the fluid becomes green, then yellow, and sometimes dark brown.

To separate glycogen from the urine, it is evaporated to a syrup, and potassium hydrate and absolute alcohol added until a cloud, due to the separation of the potassium salts, is obtained. The fluid is decanted, and the precipitate washed several times with absolute alcohol. It is then dissolved in acetic acid, and reprecipitated with absolute alcohol. The purified precipitate is warmed with alcohol and dried. A white powder, soluble in water, giving a brown colour with iodine, and slowly reducing Fehling's solution is obtained.

Alkaptonuria.—In this condition the urine, when fresh, is acid in reaction, and of a normal colour. On standing it rapidly darkens, commencing at the surface, and passes through various shades of brown to absolute blackness, owing to absorption of oxygen from the air. The change of colour takes place more quickly if the urine is made alkaline. Linen and woollen fabrics moistened with the urine are stained brown or black, and it is by this staining of the linen that attention is often drawn to the condition. On heating the urine with Fehling's solution a deep-brown colour develops, and a copious reduction occurs, but the browning of the liquid, in which the orange precipitate is suspended, gives to the test a peculiar appearance which distinguishes

it from the ordinary reduction by the sugars. An ammoniacal solution of silver nitrate is rapidly reduced even in the cold. On heating the urine with Nylander's solution, it is at once darkened by the alkali of the reagent, but no reduction of the bismuth occurs. The urine is optically inactive, does not ferment with yeast, and does not yield a crystalline osazone with phenylhydrazin. The most striking reaction is produced by adding a dilute solution of ferric chloride to the urine, drop by drop. The addition of each drop produces a deep blue colour, which lasts for only a moment, but is repeated until oxidation is complete.

The characteristic reactions of the urine are due to the presence of homogentisic acid (para-di-oxy-benzene-acetic acid or hydro-quinone-acetic acid, $C_6H_3.(OH)_2.CH_2.COOH$).

This may be isolated by heating the urine to boiling, and adding 5 grams of solid neutral lead acetate for each 100 c.c. The dense precipitate that forms is filtered off while the urine is still hot, and the clear yellow filtrate is put aside in a cool place to stand for twenty-four hours. The crystalline lead compound of homogentisic acid that separates out is filtered off, washed, and dried. The free acid may be recovered by dissolving the powdered lead homogentisate in ether, and decomposing it with a stream of sulphuretted hydrogen. The filtrate from this is allowed to evaporate, and the colourless crystals of homogentisic acid, with a melting-point of 146° to 147° C., are left.

In the routine examination of urines for sugar different observers employ different preliminary tests. In this country Fehling's, and on the Continent Trommer's, is the one most commonly used ; but some authors recommend Nylander's reagent, as they contend that it keeps well, and does not give a reaction with many of the disturbing substances that reduce alkaline solutions of copper. Others strongly advocate Crismer's safranin test, for they point out that, while it is not affected by creatinin, uric acid, and other reducing substances occurring in normal urines, it is very delicate. Although its extreme delicacy is a drawback, since many normal urines give a slight reaction, it has the compensating advantage that, should the test be negative, the presence of even a trace of sugar is excluded, and there is no need to proceed further. If it is positive, the result must always be confirmed by other methods. In my own work, as I have already mentioned, I have for some time been regularly using Benedict's test, with very satisfactory results. The solution keeps well, it is sufficiently delicate to reveal any pathological excess of sugar, but does not react with normal urines, and is not reduced by most of the substances giving rise to difficulties when Fehling's solution is employed. Whichever

(I) Reduction Test

Negative

Slight

Marked and symptoms of diabetes — Dextrose (levulose)

(II) Phenylhydrazin Test

Negative

Positive

Uric Ac., Creatinin, &c.
Homogentistic acid

Characteristic crystals

M P—about 150° C.   M.P—about 200° C.

Pentose
Isomaltose

Dextrose
Levulose
Maltose

Scanty short cryst,
increased when urine
warmed with H_2SO_4

Glucuronic acid

{ Globules with
short spines }

Lactose

(III) Fermentation Test

Positive

(IVb) Polariscope

Levo rotatory

Levulose

Dextro rotatory

Rotation and reduction
unchanged

Dextrose

(Vb) Hydrolise dil. H_2SO_4

{ Dextro-rotation
diminished,
reduction
increased }

Maltose

Negative

(IVa) Fehling test with
fermented urine

Still reduces

(Va) Phloroglucin test

Positive

No spectrum   Spectrum

Lactose

(VI) Orcin Test (Bial)

Negative   Positive

Glucuronic Ac.   Pentose

Negative

No reduction

Dextrose
(under 0·1 per cent.)

Negative

Isomaltose

test is selected, it is important that its failings should be carefully borne in mind, and that it should be constantly practised, for it is only by practice that doubtful results can be satisfactorily explained, and mistakes in diagnosis be guarded against.

A well-marked reduction with any of the preliminary tests associated with the symptoms of diabetes leaves little doubt that dextrose, and possibly also levulose, is present. If only a slight, or ambiguous, reaction is obtained the result must be confirmed, and for this the fermentation and phenylhydrazin tests are the best. By then proceeding in the manner indicated in the preceding pages, and outlined in the table (p. 74), it is possible to determine the particular substance to which the reduction is due. A complete investigation is advisable in the more marked cases also ; for, although this may not be necessary for diagnostic purposes, it is probable that much-needed light would be thrown on the urinary changes in diabetes, and allied conditions, if more exhaustive routine analyses of the urine were made.

BIBLIOGRAPHY

Abeles, *Centralb. f. d. med. Wissensch.*, 1879.
Allen, *Lancet*, 1894 ; *Chemistry of the Urine*, 1895 ; *Commerc. Org. Analysis*, 1909, i.
Allen and Tollens, *Liebig's Ann.*, 1890.
Baisch, *Zeit. f. phys. Chem.*, xix., xx.
Barfoed, *Journ. f. prakt. Chem.*, 1872 ; *Zeit. f. analytic. Chem.*, xii.
Bauer, *Zeit. f. phys. Chem.*, 1907.
Benedict, *Journ. Biolog. Chem.*, 1909 ; *Journ. Amer. Med. Assoc.*, 1911.
Bial, *Deut. med. Woch.*, 1902, 1903.
Binet, *Rev. d. l. Suisse romande*, 1892.
Borchardat, *Zeit. f. phys. Chem.*, 1908.
Böttger, *Zeit. f. prakt. Chem.*, lxx. ; *Chem. Centralb.*, 1857.
Brücke, *Wiener med. Woch.*, 1858 ; *Sitzungsber. d. k. Akad. d. Wiss. z. Wien*, 1875.
Brückner, *Aertzliche Rundschau*, 1899.
Bruel, *Arch. f. exp. Path.*, 1898.
Cammidge, *Tr. Med. Chi. Soc.*, lxxxviii.
Crismer, *Pharm. Zeit.*, xxxiii. ; *Pharm. Journ.*, xix.
Fischer, *Berich. d. Deut. Chem. Gesellsch.*, 1884.
Fluciger, *Zeit. f. physiolog. Chem.*, 1885.
Friedlander, *Arch. d. Heilkunde*, vi.
Geelmuyden, *Jahresberich. f. Tierch.*, 1903.
Haas, *Centralb. f. d. med. Wissensch.*, 1876.
Heller, *Dessen. Archiv.*, 1844.

Hoppe-Seyler, *Zeit. f. phys. Chem.*, 1892.

Jacoby, *Charité Annalen*, 1898.

v. Jaksch, *Zeit. f. klin. Med.*, xi.

Johnson, *Lancet*, 1894 ; *Tr. Pharmaceut. Journ.*, 1895 ; *Proc. Roy. Soc.*, xliii. ; *Tr. Roy. Med. Chi. Soc.*, lxxvi.

Jolles, *Centralb. f. inn. Med.*, 1903.

Jones, Bence, *Quarterly Journ. Chem. Soc.*, xiv.

Kellas and Wethered, *Lancet*, 1906.

Kowarski, *Berl. Klin. Woch.*, 1899, 1900.

Külz, *Pflüger's Arch.*, xiii.

Langstein and Steinitz, *Biochem. Zeit.*, 1906.

Levesson, *Biochem. Zeit.*, 1907.

Leuken, *Apoth. Zeit.*, i.

Lowenstein, *Allg. med. Centralzeit.*, 1900.

Lohnstein, *Pflüger's Arch.*, 1896 ; *Chem. Zentralb.*, 1896, 1899.

Luther, *Chem. Centralb.*, 1891.

Maclean, *Brit. Med. Journ.*, 1907.

Malay, *Wiener Akad. Sitzungsber.*, lxiii.

Malfatti, *Centralb. f. Harn. u. Sexualorg.*, 1905.

Maschke, *Zeit. analytic. Chem.*, 1877.

Mayer, *Zeit. f. phys. Chem.*, 1901.

Mayer and Neuberg, *Zeit. f. physiol. Chem.*, 1900.

Meissner and Babo, *Zeit. f. Ration. Med.*, ii.

Molisch, *Monatschr. d. Chem.*, vii.

Moore, *Lancet*, 1844.

Moritz, *Deut. Arch. f. klin. Med.*, 1890.

Mulder, *Arch. f. d. holländ. Beitrage*, 1861–2.

Neubauer and Strauss, *Zeit. f. phys. Chem.*, xxxv., 1902.

Neuberg and Wohlgemuth, *Zeit. f. phys. Chem.*, xxxv., 1902.

Neumann, *Berl. klin. Woch.*, 1904.

Neumayer, *Deut. Arch. f. klin. Med.*, 1900.

Nylander, *Zeit. f. physiol. Chem.*, 1883–4.

Pavy, *Guy's Hosp. Rep.*, xxi. ; *Physiol. of Carbohydrates*, 1894 ; *Carbohydrate Metabolism and Diabetes*, 1906.

Pinoff, *Virchow's Arch.*, cvii.

Porcher and Nicholas, *Journ. d. Physiol.*, 1901.

Purdy, *Pract. Uranalysis*, 1894.

Quinquand, *C. R. d. Soc. d. Biol.*, 1889.

Riegler, *Deut. med. Woch.*, 1901, 1903.

Roos, *Zeit. f. phys. Chem.*, xv.

Rosenberger, *Zeit. f. phys. Chem.*, 1906.

Rubner, *Zeitschr. f. Biol.*, 1884.

Salkowski, *Zentralb. f. med. Wissensch.*, 1892 ; *Zeit. f. phys. Chem.*, 1879, 1899 ; *Berl. klin. Woch.*, 1905.

Salkowski and Blumenthal, *Charite Annalen*, 1898.

Schondroff, *Pflüger's Arch.*, 1908.

Schoorl and Kalmthout, *Chem. Berich.*, 1906.

Seegen, *Pflüger's Arch.*, lxiv.

Soxhlet, *Journ. f. prakt. Chem.*, xxi.

Tollens, *Chem. Berich.*, 1896 ; *Zeit. v. deut. Zuckerind.*, 1908.

Trommer, *Ann. d. Chem. u. Pharm.*, 1841.

Tuchen, *Virchow's Arch.*, xxvii. ; *Zeit. f. physiol. Chem.*, 1888.

Wedenski, *Zeit. f. physiol. Chem.*, xiii.

Wender, *Pharm. Post*, xxvi. ; *Analyst*, xviii.

Wheeler and Tollens, *Chem. Ber.*, 1889 ; *Lieb. Ann.*, 1889.

Wöhle, *Zeit. f. anal. Chem.*, 1904.

Worm-Müller, *Pflüger's Arch.*, xvi., xvii., xxii., xxvii.

Worms, *Bull. d. l'Acad. d. Méd. d. Paris*, 1895.

Zunz, *Journ. Méd. de Bruxelles*, 1902.

CHAPTER III

HAVING discovered that a patient is passing sugar in the urine the first point to settle is the gravity of the case. This is determined partly by the kind of sugar present. If lactose alone is found, the glycosuria is only a temporary one, and will cease when the condition giving rise to it is removed ; a pentose may be of alimentary origin, or be evidence of an inherent defect of metabolism which, although likely to be of a permanent nature, is not of serious import ; the presence of dextrose, with or without some other sugar, indicates a metabolic defect which is always of serious significance, and shows that the patient is either actually, or potentially, in a condition that is likely to result in a fatal train of symptoms. The prognosis in any particular case of dextrosuria depends upon (1) the intensity of the glycosuria, (2) the presence and degree of secondary abnormalities of metabolism, and (3) the extent to which nitrogenous equilibrium is interfered with. To determine these points a quantitative analysis of the urine for (a) sugar, (b) acetone bodies, (c) total nitrogen is necessary. In this chapter we shall consider only the methods employed, leaving for subsequent consideration the interpretation of the results obtained.

A quantitative analysis should never be made with an odd sample of urine, as the results are apt to be most misleading. The whole excretion for twenty-four hours should be collected, and carefully measured, starting, for example, with that passed after 8 A.M., and continuing to collect until, but not including, all up to 8 A.M. next day. A sample from the mixed excretion having been analysed, the results may then be worked out for the total twenty-four hours' specimen.

(a) *The reducing sugars* in the urine are commonly estimated by titration with an alkaline solution of copper, but an alkaline solution of a mercury salt, estimations with the polariscope, and by fermentation may also be employed. In special cases (*e.g.* the pentoses, &c.) other methods are also made use of.

When sugar is known to be present in considerable quantity,

a rough estimate of the amount may be arrived at by Naunyn's table :—

A daily excretion of 2 litres and a sp. gr of 1·028–1·030 = about 2–3 %
     ,,   ,,   3   ,,   ,,   1·028–1·032 =   ,,   3–5   %
     ,,   ,,   5   ,,   ,,   1·030–1·035 =   ,,   5–7   %
     ,,   ,,   6–10 ,,   ,,   1·030–1·042 =   ,,   6–20 %

The percentage of sugar in the urine can also be approximately determined from the quantity and specific gravity, by deducting from the specific gravity as much as would represent the specific gravity of a normal urine diluted to the same amount as that passed by the patient, and multiplying the difference by 230. Thus if a diabetic passes 3 litres of urine with a specific gravity of 1·030, and it is assumed that a normal person passes 2 litres with a specific gravity of 1·015 in the twenty-four hours, the latter on being diluted to 3 litres would have a specific gravity of 1·010.

$$\frac{2 \times 1·015 + 1·000}{3} = 1·010$$

Deducting this from the specific gravity of the diabetic urine (1·030 − 1·010) = 0·020, and (0·020 × 230) = 4·6, so the urine would contain about 4·6 per cent. of sugar. Such a calculation is not, however, quite justifiable, for it assumes that the change in specific gravity is entirely due to dextrose ; but this is not the case, since alterations in the excretion of urea and inorganic salts may, and generally do, occur, and so influence the result.

I. Titration with Alkaline Solutions of Copper

1. **Fehling-Soxhlet Method.**—This may be used either volumetrically or gravimetrically. The latter is more accurate, but the volumetric method is generally employed for clinical purposes, as it is simpler and is more rapid. The duration of the operation, partial re-oxidation of the precipitate, &c., are apt to introduce errors, but with care and experience these disadvantages can be minimised, and any unavoidable errors are probably more than counterbalanced by errors in collecting the urine and working out the daily output.

(i) Volumetric.—The estimation is based upon an exact decoloration of a known volume of Fehling's solution by a measured volume of the urine, which should be diluted to contain approximately 1·0 per cent. of reducing sugar. Dilution of the urine tends to eliminate the effect of the normal reducing substances, and is particularly necessary when a high percentage of sugar is

present, since the theoretical reduction only takes place when the solution contains between 0·5 and 1 per cent. ; above the latter point there may be an error of 2 per cent. The greater the amount of sugar, therefore the higher must be the dilution. The necessary dilution may be roughly guessed at from the specific gravity of the specimen. Thus with a specific gravity of 1·025, or under, the urine should be diluted five times, and with a specific gravity of 1·030, or over, ten times. Urines containing less than 0·5 per cent. of sugar cannot be satisfactorily titrated with Fehling's solution unless, as Hammerstein suggests, a weighed quantity of dextrose sufficient to allow of a ten times dilution is added, and allowed for in the subsequent calculations. If there is less than 0·5 per cent. of sugar the fermented and unfermented urine may both be titrated, and the difference in the result taken to represent the fermentable sugar.

Any albumen that may be present should be removed by acidifying, boiling, and filtering before the titration is commenced, as it interferes with the settling of the precipitate. Allowance must of course be made for any alteration in volume caused by the process.

In carrying out the titration 10 c.c. of Fehling's solution (p. 30) are generally used. This amount is completely reduced by 0·05 gram of dextrose in 1 per cent. solution. Other sugars give slightly different figures,

$$
\text{10 c.c. of Fehling being reduced by}
\begin{cases}
0\text{·}0500 \text{ gram of dextrose} \\
0\text{·}0543 \text{ gram of levulose} \\
0\text{·}0511 \text{ gram of galactose} \\
0\text{·}0678 \text{ gram of lactose} \\
0\text{·}0807 \text{ gram of maltose} \\
0\text{·}0430 \text{ gram of pentose (arabinose)}
\end{cases}
$$

Knowing the dilution of the urine (d), and the number of cubic centimetres (x) required to effect complete reduction of the 10 c.c. of Fehling, the weight per litre of sugar present in the original urine is calculated as follows :—

$$
\frac{0\text{·}05 \text{ (\&c.)} \times 1000 \times d}{x} = \text{grams per litre}
$$

From this the total daily output is arrived at by multiplying the result by the measured volume for the twenty-four hours, expressed in litres and decimals of a litre. If it is preferred to obtain the result in grains per fluid ounce, it is only necessary to remember that 1 gram = 15·5 grains approximately, and that a fluid ounce = 28·4 c.c.

For accurate work the Fehling solution must be standardised by being titrated against a solution of dextrose containing exactly 5 grams per litre, or, since pure cane-sugar is more easily obtained than pure dextrose, with a solution of invert sugar of known strength.

To prepare a standard solution of invert sugar dissolve 4·75 grams of pure crystallised cane-sugar, that has been dried at 100° C., in 75 c.c. of distilled water, and add 5 c.c. of pure hydrochloric acid (sp. gr. 1·188). Place the mixture in a water-bath at 70° C., and keep at that temperature for ten minutes. Cool rapidly to 20° C., exactly neutralise the acid with sodium hydrate, and dilute to 1000 c.c. with distilled water. Every 10 c.c. of this solution contains 0·05 gram of dextrose, and should reduce 10 c.c. of Fehling solution completely. If the Fehling solution is too strong the necessary dilution can be determined from the following equation :—

$$\frac{N \times d}{n} = C$$

Where $\begin{cases} C = \text{the number of c.c. of water to be added to the remaining solution.} \\ N = \text{the number of c.c. of solution remaining after the titration.} \\ n = \text{the number of c.c. consumed in one titration.} \\ d = \text{the difference between the number of c.c. theoretically required, and actually consumed, in the titration.} \end{cases}$

To estimate the amount of sugar in a given specimen of urine the procedure is as follows :—

By means of a graduated pipette, 5 c.c. of Fehling's solution " A," and 5 c.c. of solution " B," are carefully measured, and mixed, in a small flask. The mixture is then diluted with 40 c.c. of distilled water, a few fragments of clay-pipe stem are introduced to prevent bumping, and it is boiled on a wire-gauze, or sand-bath. The urine, which has been previously diluted if necessary, is run in from a burette, adding 1 or 2 c.c. at a time at first, and boiling between each addition. Later, as the end-point is neared, the urine is added drop by drop, gently boiling meanwhile. The end of the reaction is reached when, on removing the flask and allowing the cuprous oxide precipitate to settle, the supernatent fluid is no longer blue when viewed against a white surface. The result obtained with this first estimation will, however, be only approximate, as a portion of the suboxide dissolves in the ammonia liberated by the prolonged boiling of the urine and becomes re-oxidised. It is therefore desirable to carry out a second titration, pouring the approximate amount of urine required to effect reduction into the Fehling solution to start with, boiling for two minutes, removing the flame, and examining the clear layer of fluid just below the meniscus. This is most satisfactorily accomplished by looking through the flask with the eye on a level with the meniscus against a piece of white paper held in front of a window. If the solution is found to be

still slightly blue, or the precipitate is slow in settling and a clear line does not quickly form, an insufficient quantity of urine has been added, or it has not been sufficiently diluted, and a third titration must be performed, using a little more urine, or a more dilute specimen. If, on the other hand, the fluid has a yellow tinge, too much sugar is present, the excess having given Moore's test, and another estimation with a smaller quantity of urine must be carried out. By a series of trials in this way the amount of urine required to at once decolorise 10 c.c. of Fehling's solution may be determined. (Soxhlet's modification.)

Various other methods of using Fehling's solution for the estimation of sugar in the urine have been proposed, some of them depending upon the number of drops of urine required to de-colorise the solution, but none of them are as accurate as that just described, and many give most unreliable results.

The chief practical difficulty in carrying out volumetric estimations of sugar with Fehling's solution is the determination of the end-point. The colour of the mass of the fluid cannot be relied upon, as it is full of the red precipitate of copper oxide, and to attempt to remove this by filtration vitiates the result, since it is partially dissolved by the ammonia present, and is re-oxidised on contact with the air to the blue cupric salt. The fluid cannot be allowed to stand too long for the precipitate to settle, as the yellow hydroxide deposited on the glass gives to it the comple-mentary colour, blue, and, moreover, re-oxidation from the air is very rapid. For the latter reason it is also necessary to carry out the titration as quickly as possible, and it is advisable to con-duct it in a flask, in an atmosphere of steam, so that the influence of the atmospheric oxygen may be avoided as much as possible. It has been suggested that the end-point should be determined by touching a piece of filter-paper soaked in a dilute solution of potassium ferrocyanide with a drop of the solution, or filtering a few drops of the liquid through a small filter into a mixture of acetic acid and dilute potassium ferrocyanide contained in a porce-lain crucible or placed on a white plate. If copper is still present a more or less marked brown coloration will be given at once. At times, however, it is difficult to separate the hydroxide pre-cipitate effectually by filtration, and a doubtful result is obtained in consequence. Ling, Rendle, and Jones have suggested ferrous thiocyanate as the indicator, and do not filter the solution. When a drop of this reagent is brought into contact with a drop of the solution, removed with a glass rod, it gives the characteristic red colour of ferric thiocyanate if any unreduced cupric salt is still present.

The ferrous thiocyanate indicator is prepared by dissolving 1 gram of ferrous ammonium sulphate, and 1·5 grams of ammonium thiocyanate, in 10 c.c. of water at 120° C., and immediately cooling, 2·5 c.c. of concentrated hydrochloric acid are then added. The resulting brown solution is decolorised by adding a trace of zinc dust. On keeping, the brown colour returns, but may be removed with a little more zinc dust. After being decolorised several times the delicacy of the reagent is reduced, but as it is at first too delicate it is best to prepare it the day before it is required, and use it after the second decolorisation.

It has been proposed to facilitate the separation of the precipitate by the addition of pure calcium carbonate, or calcium chloride (10 per cent.), to the Fehling solution, but most observers find that they offer no great advantage.

Various modifications of Fehling's method have been introduced with a view to rendering the end reaction more definite. Among these may be mentioned—

(a) *Gerrard's Cyanide Process.*—Depends upon the formation of a colourless double cyanide of potassium and copper, when a solution of potassium cyanide is added to Fehling's solution. This cyanide has the power of holding in solution any cuprous oxide formed by the reduction of an excess of Fehling's solution by any sugar present, so that the end of the reduction is indicated by decolorisation of the fluid, but without the formation of a precipitate.

Ten c.c. of freshly prepared Fehling's solution are diluted to 50 c.c. with water, and boiled. To the boiling liquid is then added a 5 per cent. solution of potassium cyanide, with constant stirring, until the blue colour is just discharged, carefully avoiding an excess. Another 10 c.c. of Fehling are then introduced, and the urine is rapidly run in from a burette, boiling, and stirring the liquid meanwhile, until the blue colour is just discharged. Only the second 10 c.c. of Fehling undergoes reduction and is reckoned in the calculation. This process is strongly recommended by Allen, but it is not easy to add the exact amount of potassium cyanide required, and, moreover, objectionable cyanide fumes escape during the heating process.

(b) Williamson and others avoid the difficulty of the end-reaction by adding potassium ferrocyanide. As the ferrocyanide itself exerts some reducing action on the copper solution, very accurate results are not possible.

Williamson's method is carried out as follows : 10 c.c. of Fehling's solution are diluted with 20 c.c. of distilled water, and 4 c.c. of a $\frac{1}{20}$ per cent. solution of potassium ferrocyanide added. The diluted urine is then run, drop by drop, into the boiling mixture until the blue colour has entirely disappeared.

Repiton uses potassium ferrocyanide with Pasteur's copper solution

(136 grams of sodium hydroxide, 80 grams of potassium hydroxide, and 105 grams of tartaric acid, in 500 c.c. of water ; and 45 to 48 grams of crystallised copper sulphate in 500 c.c. of water). To 5 c.c. of this solution is added 0·2 gram of potassium ferrocyanide, and the mixture is heated to boiling. The urine is then added, drop by drop, until the blue disappears, and is replaced by a golden-yellow coloration. He states that the ratio of dextrose required to reduce this solution as compared with the ordinary copper solution is 82 : 100.

(c) *Dreschel's gaunin method* (Klimmer) is based upon the fact that, in the presence of gaunin, the red suboxide forms a less readily oxidisable compound of a white colour. It gives a sharp end-reaction, but is only satisfactory with pure gaunin, which is very expensive.

A solution of gaunin, containing 0·00755 gram per c.c. is prepared by dissolving 9·375 grams of gaunin hydrochloride in 1000 c.c. of a 1 per cent. solution of sodium hydrate : 15 c.c. of this solution are added to 10 c.c. of Fehling's solution, and the mixture diluted with 25 c.c. of distilled water. This is then titrated with the diluted urine in the usual way. If the reduction produces a red colour the titration must be repeated with the addition of more gaunin to the Fehling solution.

(d) *Bang's method* is now very largely used for clinical work, as it has a definite end-point ; is rapid and reliable. To obtain good results, however, it is essential that the directions should be followed in every detail, particularly as regards the purity and concentration of the components of the standard solutions, the temperatures at which they are dissolved and mixed, the time and method of boiling, &c. Bang's method is based upon the fact that cuprous oxide, in the presence of potassium thiocyanate, forms cuprous thiocyanate, if the solution contains only alkaline carbonates, and no alkali hydroxide. The excess of copper not reduced is determined by converting it into cuprous thiocyanate with hydroxylamine.

Two solutions are prepared—(A) 500 grams of potassium carbonate, 100 grams of potassium hydrogen carbonate, and 400 grams of potassium thiocyanate are dissolved in about 1200 c.c. of distilled water at 50° to 60° C., and cooled to 30° C. Exactly 25 grams of pure copper sulphate $(CuSO_4 + 5H_2O)$, previously dissolved in 150 c.c. of hot distilled water, and cooled, are then added. After standing for twenty-four hours, the mixture is made up to 2000 c.c. and filtered. (B) 200 grams of potassium thiocyanate, dissolved in about 1500 c.c. of distilled water, and 6·55 grams of hydroxylamine sulphate, dissolved in about 300 c.c. of water, are mixed together. The mixture is then made up to 2000 c.c. This solution must be carefully standardised, and be titrated against the " A " solution. It must be kept in a dark-coloured bottle with a few grains of thymol added to prevent the growth of fungi.

To carry out the estimation 50 c.c. of the copper solution (A) are placed in a 200 c.c. Jena-glass flask, and 10 c.c. of the urine, or less made up to 10 c.c. with water, are accurately measured out with a pipette and added. The mixture is then heated to boiling on a wire-gauze (*not* a sand-bath, or asbestos board), and boiled for exactly three minutes. The solution must remain of a distinct blue tint; if it is completely decolorised the experiment must be commenced afresh, using a smaller quantity of urine. The flask is now rapidly cooled to the room temperature in a stream of cold water. The hydroxylamine solution (B) is then run in until the solution is decolorised and the last trace of green disappears, leaving only a yellow tint when the flask is held over a white slab or paper. Since 50 c.c. of the copper solution (A) correspond to about 60 mg. of sugar (50 mg. of dextrose = $0\cdot1376$ grams of copper), and the amount of unreduced copper is shown by the quantity of hydroxylamine solution (B) that is used, the quantity of sugar present in the urine taken may be calculated, but as experiment has shown that the relation is not quite constant for different strengths it is better to employ an empirical table. (See p. 86).

If the urine contains $0\cdot6$ per cent., or over, of sugar the 50 c.c. of copper solution will be completely decolorised before the mixture has been boiled for the full three minutes, so that it should be previously diluted according to the following table :—

With a sugar-content of $0\cdot0$–$0\cdot5$ % use 10 c.c. of urine

,,	,,	$0\cdot5$–$1\cdot0$,,	,,	5 c.c.	,,	and $5\cdot0$ c.c. water
,,	,,	$1\cdot0$–$2\cdot5$,,	,,	2 c.c.	,,	and $8\cdot0$ c.c. ,,
,,	,,	$2\cdot5$–$6\cdot0$,,	,,	1 c.c.	,,	and $9\cdot0$ c.c. ,,
,,	,,	over $6\cdot0$,,	,,	$0\cdot5$ c.c.	,,	and $9\cdot5$ c.c. ,,

The degree of dilution necessary can usually be estimated fairly accurately from a consideration of the specific gravity, and the rate at which the copper is reduced in the qualitative tests. Albumen does not interfere with the reaction, and need not therefore be removed.

(*e*) Benedict has modified his qualitative test solution for use in quantitative work by adding potassium sulphocyanate and potassium ferrocyanide, with the result that the reduced copper is precipitated as cuprous sulphocyanate, a snow-white compound, which is rather an aid than a hindrance to accurate observation of the disappearance of the last trace of blue colour.

The solution for quantitative work has the following composition :—

	grams or c.c.
Copper sulphate (pure crystallised)	$18\cdot0$
Sodium carbonate (crystallised)[1]	$200\cdot0$
Sodium, or potassium, citrate	$200\cdot0$
Potassium sulphocyanate	$125\cdot0$
Five per cent. potassium ferrocyanide solution . . .	$5\cdot0$
Distilled water to make a total volume of	$1,000\cdot0$

[1] One-half the weight of the anhydrous salt may be used.

Bang's Method

C.c. of Bang, "B."	Mg. of Sugar.	C.c. of Bang, "B."	Mg. of Sugar.	C.c. of Bang, "B."	Mg. of Sugar.
0·75	60·0	19·00	31·4	37·50	10·9
1·00	59·4	19·50	30·8	38·00	10·4
1·50	58·4	20·00	30·2	38·50	9·9
2·00	57·3	20·50	29·6	39·00	9·4
2·50	56·2	21·00	29·0	39·50	9·0
3·00	55·0	21·50	28·3	40·00	8·5
3·50	54·3	22·00	27·7	40·50	8·1
4·00	53·4	22·50	27·1	41·00	7·6
4·50	52·6	23·00	26·5	41·50	7·2
5·00	51·6	23·50	25·8	42·00	6·7
5·50	50·7	24·00	25·2	42·50	6·3
6·00	49·8	24·50	24·6	43·00	5·8
6·50	48·9	25·00	24·1	43·50	5·4
7·00	48·0	25·50	23·3	44·00	4·9
7·50	47·2	26·00	22·9	44·50	4·5
8·00	46·3	26·50	22·3	45·00	4·1
8·50	45·5	27·00	21·8	45·50	3·7
9·00	44·7	27·50	21·2	46·00	3·3
9·50	44·0	28·00	20·7	46·50	2·9
10·00	43·3	28·50	20·1	47·00	2·5
10·50	42·5	29·00	19·6	47·50	2·1
11·00	41·8	29·50	19·1	48·00	1·7
11·50	41·1	30·00	18·6	48·50	1·3
12·00	40·4	30·50	18·0	49·00	0·9
12·50	39·7	31·00	17·5	49·50	0·5
13·00	39·0	31·50	17·0	50·00	0·0
13·50	38·3	32·00	16·5	...	...
14·00	37·7	32·50	15·9	...	...
14·50	37·1	33·00	15·4	...	...
15·00	36·4	33·50	14·9	...	...
15·50	35·8	34·00	14·4	...	...
16·00	35·1	34·50	13·9	...	...
16·50	34·5	35·00	13·4	...	...
17·00	33·9	35·50	12·9	...	...
17·50	33·3	36·00	12·4	...	...
18·00	32·6	36·50	11·9	...	...
18·50	32·0	37·00	11·4	...	...

With the aid of heat dissolve the carbonate, citrate, and sulpho-cyanate in enough water to make about 800 c.c. of the mixture, and filter if necessary. Dissolve the copper sulphate separately in about 100 c.c. of water, and pour the solution slowly into the other liquid, with constant stirring. Add the ferrocyanide solution, cool, and dilute to exactly one litre. Of the various constituents, the copper salt only need be weighed with exactness. Twenty-five c.c. of the reagent are reduced by 50 mg. of glucose.

Sugar estimations are conducted as follows : The urine, 10 c.c. of

which should be diluted with water to 100 c.c. (unless the sugar-content is believed to be low), is poured into a 50 c.c. burette up to the zero mark. Twenty-five c.c. of the reagent are measured with a pipette into a porcelain evaporation dish (25 to 30 cm. in diameter), 10 to 20 grams of crystallised sodium carbonate (or one-half the weight of the anhydrous salt) are added, together with a small quantity of powdered pumice stone, or talcum, and the mixture heated to boiling over a free flame until the carbonate has entirely dissolved. The diluted urine is now run in from the burette, rather rapidly, until a chalk-white precipitate forms, and the blue colour of the mixture begins to lessen perceptibly, after which the solution from the burette must be run in a few drops at a time, until the disappearance of the last trace of blue colour, which marks the end-point. The solution must be kept vigorously boiling throughout the entire titration. If the mixture becomes too concentrated during the process, water may be added from time to time to replace the volume lost by evaporation. The calculation of the percentage of sugar in the original sample of urine is very simple. The 25 c.c. of copper solution are reduced by exactly 50 mg. of glucose. Therefore the volume run out of the burette to effect the reduction contained 50 mg. of the sugar. When the urine is diluted 1 : 10, as in the usual titration of diabetic urines, the formula for calculating the percentage of sugar is the following—

$$\frac{0 \cdot 050}{x} \times 1000 = \text{per cent. in original sample,}$$

where x is the number of c.c. of the diluted urine required to reduce 25 c.c. of the copper solution.

In this method chloroform must not be present during the titration. If used as a preservative in the urine it may be removed by boiling a sample for a few minutes, and then diluting to its original volume.

This reagent, like that employed for qualitative work, will keep indefinitely. Benedict states that repeated determinations, and comparisons of the results with those obtained by the polariscope, and by Allihn's gravimetric process, have shown the method to be probably more exact than any other titration method available for sugar work.

(*f*) *Pavy's Ammoniacal Copper Method.*—This modification of Fehling's solution is based upon the fact that, in the presence of a sufficient excess of ammonium hydroxide, the cuprous oxide is not precipitated, but forms a colourless solution, so that the end of the reaction is indicated by decolorisation of the blue fluid.

As the ammoniacal cuprous solution is very readily oxidised, it is necessary to prevent access of air during the titration. This may be done by attaching the nozzle of the burette containing the urine to a tube passing through an indiarubber stopper closing the flask that contains the copper solution. A second tube conveys the steam and ammonia gas generated into a flask of cold water. To prevent the

tendency to "suck back," the delivery end of this tube should dip below the surface of a little mercury at the bottom of the flask. A better arrangement is to have a third tube in the stopper by which a slow current of hydrogen, or coal-gas, is passed through the flask containing the boiling copper solution. When coal-gas is used a red film of cuprous acetylide is apt to form on the surface, but can be disregarded.

To prepare the ammoniacal Pavy's solution 120 c.c. of Fehling's solution are mixed with 300 c.c. of strong ammonia (sp. gr. 0·880), and 400 c.c. of a 12 per cent. solution of sodium hydroxide (sp. gr. 1·14). The mixture is then made up to 1000 c.c. One hundred c.c. of this solution corresponds to 10 c.c. of Fehling's solution—that is to say, is reduced by 0·05 gram of dextrose.

To carry out the titration 100 c.c. of the solution are placed in a flask, a few fragments of pumice, or pipe-stem, added, the burette and tubes adjusted, and the liquid brought to boiling. The diluted urine is then run in from the burette until the blue colour of the liquid has entirely disappeared, the boiling being meanwhile continued regularly. The end-reaction is very sharply marked, and may be confirmed by bubbling air through the liquid directly decolorisation is complete. The blue colour should reappear in a few seconds unless too much urine has been used, when a longer time will elapse. Reduction occurs much more slowly than with Fehling's solution, and Pavy's solution has a different oxidising power for maltose, lactose, and invert sugar. The practical disadvantages of this method are the inconvenience of working with the ammoniacal solution, the great dilution of the solution as compared with Fehling's, and the necessity for using a special apparatus.

The following method of carrying out the test, which is simpler and more convenient, is sometimes used in clinical work, it is, however, less accurate: 10 c.c. of Pavy's solution are accurately measured into a wide test-tube, a few fragments of clay-pipe are dropped in, and 8 or 10 drops of petroleum, or paraffin-oil, are poured on the surface to exclude the air. The test-tube is then suspended in the neck of a wide-mouthed flask containing hot water, which is heated until the contents of the tube boil. The urine to be tested is treated with an equal volume of ammonia, and filtered from the precipitated phosphates. A known volume of the filtrate is then further diluted with a definite quantity of water, according to the proportion of sugar believed to be present. The diluted urine is added, drop by drop, to the boiling Pavy's solution from a graduated pipette, or small burette, until the disappearance of the colour indicates the termination of the reaction. The 10 c.c. of Pavy's solution used corresponds to 0·005 gram of sugar.

Healthy urine may exert a reducing action on Pavy's solution equal to a liquid containing 0·1 to 0·3 per cent. of dextrose. Of this one quarter is ascribed to uric acid (removable by lead acetate), and the remainder to creatinin (removable by mercuric chloride).

Sahli advises a more concentrated solution, which is prepared in two parts that are mixed in equal volumes as required :—

A $\begin{cases} \text{Copper sulphate cryst. 4·158 grs.} \\ \text{Distilled water to . . 500 c.c.} \end{cases}$ B $\begin{cases} \text{Sodium-potassium tartrate} & 20\cdot4 \\ \text{Potassium hydroxide} & . & 20\cdot4 \\ \text{Ammonia (sp. gr. 0·88)} & . & 300\cdot0 \\ \text{Distilled water to} & . & . & 500\cdot0 \end{cases}$

Five c.c. of each of these solutions are placed in a flask, holding about 100 c.c., and 30 c.c. of water added. The contents are heated over an asbestos board, with a micro-burner that can be regulated, so that they are gently, but not vigorously boiled. The urine, diluted so that a fairly large amount, but not a sufficient quantity to appreciably decrease the concentration of the alkali in the solution, is run in from a burette, taking care that the boiling is never interrupted, until the fluid is decolorised.

(*g*) *Purdy's Method.*—A solution prepared by mixing 4·752 grams of copper sulphate, dissolved in water, with 23·5 grams of potassium hydrate, previously dissolved in water, 350 c.c. of ammonia (sp. gr. 0·9), and 38 c.c. of pure glycerine, is made up to 1000 c.c. with distilled water. To 35 c.c. of this fluid, placed in a 150 to 200 c.c. flask, is added enough water to half fill the flask. The flask is then closed with a perforated cork. Through one hole the tip of the burette containing the urine is passed, and through another a tube bent at right angles to carry the steam and fumes away from the face. The fluid is brought to the boil, and the urine added drop by drop, until the blue colour begins to fade ; then more slowly, three to five seconds elapsing between each drop, until the blue colour completely disappears and leaves the test solution perfectly transparent and colourless. If it is boiled too long, so that too much ammonia escapes, the copper hydroxide will precipitate. If there is over 5 per cent. of sugar the urine should be diluted with three volumes of water (*i.e.* four times). Each 35 c.c. of Purdy's fluid is reduced by 0·02 gram of dextrose.

(*h*) *The iodometric method* (Lehmann) consists in heating the urine with Fehling's solution, filtering, treating with sulphuric acid and potassium iodide, and titrating the liberated iodine with sodium thiosulphate and starch. It is based on the principle that sugar precipitates a proportionate amount of the copper in the Fehling solution, and that the precipitated cuprous oxide remains behind on the filter. By the action of the sulphuric acid and potassium iodide on the filtrate, one atom of iodine is liberated for each molecule of the copper sulphate. The tedious decolorisation of the Fehling test is avoided, and the iodine reaction in its place is much plainer. Only the copper still in solution—not that already reduced— induces the liberation of the iodine, and the cuprous oxide becomes quantitatively transformed into cuprous iodide. According to Citron, the best technique is as follows :—

Ten c.c. of the potassium-sodium tartrate solution (see below) are placed in a porcelain dish. Ten c.c. of copper solution are measured out, and nearly all is poured in. The mixture is now thoroughly stirred. Then exactly 1 c.c. of urine is introduced, and, when all is dissolved, the mixture is heated over a small flame for a minute and a half, to boiling, and then cooled by placing the dish in a vessel of cold water. (While it is cooling the starch solution is prepared.) On adding 4 c.c. of a 25 per cent. solution of sulphuric acid a thick, viscid precipitate is at once thrown down. The solution of sodium thiosulphate is then run in from a burette, graduated for percentages, a line at a time, and a few c.c. of the starch solution are added. The fluid then turns deep blue, or possibly a little more of the sulphuric acid solution and starch solution may be required. More of the thiosulphate solution is then run in, a line at a time, until the fluid turns milk white, and remains so even when it is stirred. The rest of the copper solution is then poured in, and also the rinsing water. If the fluid still remains white, the percentage line on the burette is the index of the proportion of sugar in the urine. If a blue tint returns, more of the sodium thiosulphate is added, drop by drop, until a permanent milky tint results. Citron uses a special isodometric burette graduated for urine percentages, and a special pipette graduated for 1 c.c. of urine, used with a syringe with a capacity of 2 c.c.

The solutions used are (a) 34·639 grams of copper sulphate dissolved in 500 grams distilled water, to which 1 c.c. of sulphuric acid is added to prevent the formation of copper carbonate ; (b) 173 grams potassium-sodium tartrate, 50 grams sodium hydroxide, and 100 grams potassium iodide dissolved in 500 c.c. water ; (c) a one-fifth normal solution of sodium thiosulphate (49·6 grams per litre), containing also 0·1 per cent. sodium arsenate to make it keep better ; (d) a 25 per cent. solution of sulphuric acid ; and (e) the starch solution, which he makes by dissolving in half a reagent glass of water a small piece, the size of a hazel nut, of a paste preparation used by photographers. The starch solution is heated to boiling and then cooled.

If an ordinary burette is used the percentage of sugar may be calculated from the following data—1 c.c. of the thiosulphate solution $=0·0127$ gram of copper, and 10 c.c. of the copper solution$=13·9$ c.c. of the thiosulphate. If " T " be the number of cubic centimetres of thiocyanate used $(13·9 - T \times 0·0127)=$the amount of reduced copper, and from this the quantity of dextrose present can be calculated by referring to the table given on page 91.

(ii) GRAVIMETRIC ESTIMATION OF REDUCING SUGARS WITH FEHLING'S SOLUTION.—This method gives very accurate results, provided the details of the manipulation are closely attended to. It is too complicated for routine clinical use, but is necessary for careful scientific investigation.

Pflüger-Allihn Method.—34·630 grams of crystallised copper sulphate are dissolved in distilled water, and diluted to 500 c.c. A second solution containing 173 grams of sodium-potassium tartrate, and 125

grams of potassium hydroxide, dissolved in water, and made up to 500 c.c. is also prepared. These solutions are preserved separately, and are mixed in equal volumes for use. To carry out the estimation 60 c.c. of the mixture (30 c.c. of A, and 30 c.c. of B) are placed in a beaker holding about 300 c.c., diluted with 60 c.c. of water, and heated on a sand-bath to boiling. Twenty-five c.c. of the urine, diluted so as to contain not more than 1 per cent. of sugar, are then added to the actively boiling fluid, and the mixture boiled for exactly two minutes. The precipitated red oxide is immediately filtered off, and the amount of copper determined by one of the following methods :—

(a) *Reduction in Hydrogen.*—The cuprous oxide is collected on an asbestos film supported by a perforated disc, or cone, of platinum in a hard glass tube, which has been previously weighed, using suction. After all the precipitate has been transferred to the filter, it is well washed with hot water, then with alcohol, and afterwards with ether, being careful that no precipitate is left near the top of the tube, where it would be removed by the cork used during the process of reduction. After drying, the tube is connected with an apparatus supplying a current of dry hydrogen, and is gently heated until the cuprous oxide is completely reduced to the metallic state. It is then cooled in a current of hydrogen, and weighed.

(b) *Direct Weighing of Cuprous Oxide.*—Prepare a gooch crucible with an asbestos felt. First thoroughly wash the asbestos with water, to remove small particles, then follow successively with 10 c.c. of alcohol and 10 c.c. of ether, and dry the crucible and its contents for thirty minutes in a water-oven at the temperature of boiling water. Cool, and weigh. Collect the precipitate of cuprous oxide on the asbestos felt, thoroughly wash with hot water, then with alcohol, and finally with ether. Dry the precipitate for thirty minutes in a hot water-oven at the temperature of boiling water. Cool, and weigh. The weight of cuprous oxide multiplied by 0·8883 gives the weight of metallic copper.

(c) *Electrolysis.*—The metallic copper in the precipitate may also be deposited electrolytically from a nitric, or sulphuric, acid solution, on to platinum and then weighed, or it may be estimated volumetrically by the permanganate method. (See Allen's *Commercial Organic Analysis*, vol. i. p. 323, 1909.)

The weight of sugar corresponding to the amount of copper, or copper oxide, obtained by any one of these methods is calculated by the use of the following factors :—

	Dextrose.	Lactose.	Maltose.	Cane-sugar after Inversion.
Cu . . .	0·5634	·7707	0·9089	0·5395
Cu_2O . .	0·5042	0·6843	0·8132	0·4790
CuO . . .	0·4535	0·6153	0·7314	0·4308

Or from Allihn's tables.

II. Estimation with Alkaline Solutions of Mercury

Several methods of estimating sugars by their reducing action on mercuric solutions have been described, but the one that is generally used in clinical work is Knapp's method.

(a) **Knapp's Method.**—This depends upon the fact that an alkaline solution of mercuric cyanide is reduced to metallic mercury in the presence of sugar. The titration can be satisfactorily carried out in artificial light, and is applicable when only a small amount of sugar is present. The solution keeps well. It is reduced by creatin, creatinin, &c., and all albumen must be carefully removed before carrying out the titration. The urine should not contain more than 0·5 to 1·0 per cent. of sugar, and must therefore be diluted if necessary.

Knapp's solution is prepared by dissolving 10 grams of pure, dry, mercuric cyanide in water, adding 100 c.c. of sodium hydroxide solution (sp. gr. 1·145), and diluting to 1000 c.c. One hundred c.c. of this solution are equivalent to 0·202 grams of dextrose, in 0·5 per cent. solution, and 0·201 gram, in 1 per cent. solution. For practical purposes 20 c.c. of the solution may be taken as equivalent to 0·05 gram of dextrose.

Twenty c.c. of the solution are diluted with 80 c.c. of water, heated to boiling, and the urine, diluted so as to contain about 0·5 per cent. of sugar, is run in as quickly as possible, until the whole of the mercury is reduced, gently boiling all the time. As the end-point is reached the solution begins to clear, and the mercury, together with the phosphates, settles to the bottom. The final point is determined by moistening a strip of white filter-paper with the clear fluid and exposing it to the fumes of concentrated hydrochloric acid and sulphuretted hydrogen successively. If the cyanide of mercury has not been completely reduced a yellow spot will appear. As soon as the mercury has been completely reduced the reading is taken, and the number of grams per litre of sugar worked out according to the formula—

$$\frac{0·05 \times 1000 \times d}{x} = \text{grams of dextrose per litre}$$

where d = the dilution of the urine, and x = the number of c.c. consumed in the titration.

(b) *Sachsse's Method.*—The mercuric solution is prepared by dissolving 18 grams of pure, dry, mercuric iodide in a solution of 25 grams of potassium iodide, adding 80 grams of potassium hydroxide, and making up to 1000 c.c. Twenty c.c. of this solution are boiled in a porcelain basin, and the diluted urine is gradually added. The completion of the reduction is indicated when a drop of the supernatent liquid ceases to give a brown colour with a drop of a very alkaline solution of stannous chloride.

Soxhlet has shown that less dextrose is required to effect reduction

the more slowly it is added, and that the concentration of the fluid is of considerable importance. It is therefore necessary that the urine should be diluted to contain as nearly as possible 1 per cent. of sugar, and that the titration should be performed rapidly. Twenty c.c. of Sachsse's fluid are reduced by 0·66 gram of dextrose in 1 per cent. solution, and by 0·65 per cent. gram in 5 per cent. solution.

Oerum recommends that, for clinical work, the reduced mercury should be collected on a filter, washed with warm 1 per cent. hydrochloric acid, then thoroughly washed with water, dissolved in boiling nitric acid, and titrated with decinormal ammonium thiocyanate, using iron alum as an indicator, as in Volhard's method, the solution having been previously standardised with a solution of dextrose, or invert sugar, of known strength.

III. Titration with Safranine

Safranine and other dyes have been used for the quantitative estimation of sugar. It is claimed that the reagents are simple and do not deteriorate on keeping, and that the method is more particularly applicable when only small quantities of sugar are present, since the reduction due to other substances than the sugars is only about one-fourth of that found with Fehling's, Bang's, and similar solutions. As proteins do not interfere with the reaction, their preliminary removal is not necessary.

To carry out the titration equal parts of a solution of safranine (1 in 10,000) and potassium hydroxide (1 in 100) are mixed. To 2 c.c. of this mixture, or more if the quantity of sugar in the urine is large, are added a known number of drops of the urine, and the mixture is warmed in a water-bath. The amount of sugar is estimated by ascertaining the number of drops which are required to just decolorise the alkaline mixture, which can be standardised against a sugar solution of known strength. To prevent the oxidising effect of contact with the air the surface of the alkaline mixture may be covered with a layer of petroleum oil, or xylol, and the urine be introduced by a fine pointed, narrow pipette, or burette, dipping below the surface. (Hasselbalch and Lindhard.)

IV. Quantitative Fermentation Tests

1. **Differential Density Method (Robert's).**—This method is useful in clinical work, and, in inexperienced hands, is more to be relied upon than titration with Fehling's solution, but the urine must contain at least 0·5 per cent. of sugar. When carefully carried out it is accurate up to about 0·1 per cent., although inattention to detail may give rise to an error as high as 5 per cent. of the total amount. It has the advantage that it is not necessary to

remove any protein that may be present, and, moreover, the results are not affected by the reducing substances of normal urine. It is based upon the fact that fermentation reduces the high specific gravity of sugar-containing urines. By comparing the specific gravity before and after fermentation, and making use of an empirical factor, which has been found by dividing the amount of sugar ascertained by titration or polarisation by the difference in density of urines before and after fermentation, the percentage of sugar in a particular specimen may be determined. The empirical factor depends upon the particular method employed, each modification requiring a different coefficient.

The specific gravity of the urine is first carefully ascertained with a pyknometer, or urinometer, graduated to the fourth decimal place, and provided with a thermometer indicating tenths of a degree. A piece of compressed yeast, the size of a hazel nut (washed free from sugar if necessary), is added to a measured quantity, 100 to 200 c.c., of the urine. The mixture is placed in a flask, gently shaken, and loosely stoppered, or closed with a cork provided with a water, or mercury, valve. After standing at the room temperature for twenty-four hours, if but little sugar is present, or forty-eight hours, if there is much, the loss by evaporation is made up by the addition of distilled water, and the clear fluid is poured off from the yeast, which will have settled to the bottom if fermentation is complete, or it may be filtered. It is first tested with Fehling's, or Trommer's, test, to make sure that all the sugar has been fermented off, then the specific gravity is again taken, with the same precautions as before. The difference between the two readings is multiplied by 0·230, the product giving the percentage of sugar in the urine. If the temperatures at which the two readings are taken are found not to be the same, one-third of a degree of the urinometer scale must be added to, or subtracted from, the specific gravity determined after fermentation for each degree of temperature, adding for an increase of temperature, and subtracting for a decrease. It is important that the urine should be quite clear and free from yeast before the specific gravity is taken, and if simple filtration does not suffice it may be shaken up with magnesia usta, or keiselgur, and again filtered. The process of fermentation may be hastened by adding to each 100 c.c. of urine 2 grams of diacidsodium phosphate, and 2 grams of sodium-potassium tartrate, with 10 grams of yeast, and incubating at 30° to 34° C. If but little sugar is present the fermentation will then take about two to three hours. The urine in which the specific gravity is taken before fermentation should be treated in the same way, but practically it is sufficient to add 0·022 to the observed specific gravity to make up for the increased density from the added salts. In every case the urine must be perfectly fresh, as fermentation generally begins spontaneously, even after standing a short time.

2. Volumetric Determination of Carbon Dioxide Evolved.

—The volume of carbon dioxide evolved by a saccharine fluid on fermentation with yeast being proportional to the amount of fermentable sugar present, it is possible to compute the sugar in a diabetic urine by this means. Theoretically, the quantity of gas given off should be read with all the precautions necessary in gas analysis, taking into account the barometric pressure, the temperature, tension of the water vapour, &c., but the introduction of such physical details into the process is an attempt at refinement not warranted by the basic inaccuracy of the method.

(a) *Einhorn's Saccharimeter.*—One gram of yeast is carefully shaken with 10 c.c. of the urine, and the mixture poured into the bulb and upright tube of the instrument, completely filling the latter. No bubbles of air must be allowed to remain at the top of the tube, nor should any collect within the first few minutes after the apparatus is filled. It is then placed in an incubator at 30° to 34° C. for twelve hours, when the reading is taken. The scale is graduated empirically in percentages of sugar (dextrose). To prevent the escape of gas a little mercury should be poured into the bent limb of the tube. It is advisable to render the urine acid by the addition of a little tartaric acid, and to boil it for several minutes to expel any contained air or gas, subsequently cooling to the temperature of the room, before carrying out the test. When the urine is rich in sugar it must be diluted four to ten, or more, times. The yeast employed must be fresh and active. Since the volume of gas evolved is dependent upon the amount of yeast, its activity, the temperature, and many other factors, and the so-called spontaneous fermentation of the yeast may give rise to some gas, the accuracy of this method has been very seriously questioned, and it has been abandoned by many observers. There are others, however, who maintain that most of the disturbing factors can be controlled and that, with care, sufficiently reliable results for clinical work can be obtained, particularly if a blank experiment, using normal urine and the same yeast under similar conditions, is carried out at the same time.

(b) *Lohenstein's Saccharimeter* is a modification of Einhorn's instrument constructed on manometric principles, which is said to give much more accurate results. It requires a minimum amount of urine, and since the gas evolved is collected over mercury, no loss from absorption occurs as in the latter instrument. Its chief drawback is that, the readings being taken on an empirical scale, the observer is dependent upon the maker for their accuracy. The sugar estimation is carried out as follows. A small piece of yeast is rubbed up with two or three volumes of water, in a suitable vessel, to a thin paste. By means of a syringe, provided with the instrument, 0·5 c.c. of the previously boiled and cooled urine is introduced on to the surface of the mercury contained in the bulb. From 0·1 to 0·2 c.c. of the yeast paste is then added, and the stopper fitted into the neck of the bulb, so

that the hole in the side comes to lie over the corresponding hole in the neck of the bulb. The removable scale is now fitted to the tube, and the surface of the mercury is adjusted to the zero mark by slightly tilting the apparatus. It is fixed in this position by turning the stopper, so that the holes are no longer in communication. A weight is placed on the stopper, and the apparatus set aside for fermentation to take place, either at the temperature of the room (20° C.), or in an incubator at 32° to 38° C. In the latter case fermentation is complete, even with a high percentage of sugar, in from three to four hours. The apparatus may also be placed in a water-bath at a temperature of 32° to 38° C., the scale having previously been removed. When the column of mercury ceases to rise, showing that fermentation is complete, the apparatus is allowed to cool to the temperature of the room at which it was filled, and the reading taken, using the appropriate scale for the conditions under which fermentations took place. If greater accuracy is desired the percentage of sugar can be calculated according to the following formula—

$$P \frac{p - P}{15}(35 - t)$$

Where P = the reading on the scale at 35° C., p = the reading on the 20° scale, and t = the temperature previously noted at which the urine was placed in the apparatus, and to which after completion of the fermentation it has again been cooled. As the instrument only gives satisfactory results if the urine contains 1·5 per cent., or less, of sugar it is necessary to dilute with water if more is present.

V. Estimation with the Polariscope

The property possessed by dextrose and other sugars of rotating the plane of polarised light proportionally to the amount present in solution is utilised to determine the percentage of sugar in a given urine. The instrument employed for the purpose is known as a polariscope, or polarimeter. Reduced to its essentials it consists of a Nicol's prism, furnishing a beam of polarised light, and an analyser of the same structure for examining it. A tube with clear-glass ends containing the urine is placed between the polariser and analyser in the path of the polarised beam. The length of the tube is usually 100 mm., or multiples of this, and the reading is in degrees. The angle of rotation must therefore be divided by 527 to give the percentage of sugar, since the specific rotation of glucose is $[a]_D = +52·74°$, or generally for any sugar according to the formula—

$$p = \frac{100 \, a}{l \times [a]_D}$$

where p = per cent., a = the observed rotation, $[a]_D$ = the specific rotation of the sugar for sodium light, and l = the length of the

tube in decimetres. For clinical purposes, however, a modified instrument with a tube of 188·6 mm., or better, 189·4 mm., long is generally used, and the readings are taken directly in percentages of dextrose. In some instruments the rotation caused by the sugar is balanced by a compensating quartz wedge, marked with an empirical scale, and ordinary white light, such as that given by a Welsbach burner, is employed.

A cheap form of apparatus specially designed for the estimation of sugar and albumen in urine is Mitscherlich's half-shadow polariscope, with Laurent's polariser. Behind the analyser is a small telescope, and behind the polariser a semicircular plate of quartz which covers one-half of the field of vision. The presence of the quartz plate causes an alteration in phase of half a wave length, and allows light to pass even when the polariser and analyser are crossed. If the analyser is rotated so as to cause the quartz plate to become dark, light passes through the uncovered half of the field. In a position intermediate between these two the halves of the field appear equally illuminated ; this is the zero point of the instrument. The slightest deviation from this neutral position causes one-half of the field to appear darker, and the other lighter than before. Attached to the analyser is a pointer, which moves to the right or left over a metal disc divided into angular degrees, by which the readings are taken. By means of a vernier, ten divisions of which correspond to nine divisions of the disc, readings to a tenth, and estimations to a twentieth, of a degree may be made. The instrument is constructed for homogeneous light, and a sodium lamp must therefore be used as the source of illumination. Sodium chloride in a Bunsen flame is generally employed, but a fused mixture of sodium chloride and phosphate is better. With the pointer and vernier at zero, and the telescope focused on the quartz plate, the field of vision should appear as an evenly illuminate circle, divided into two halves by a vertical line. If a tube containing a saccharine fluid is now put into position between the polariser and analyser, and the apparatus is refocused, one-half of the field will appear brighter than the other, but on rotating the eye-piece until the optical disturbance produced by the introduction of the rotating fluid is compensated, equal illumination of the whole field can again be attained. The angle of rotation necessary to effect this indicates the rotatory power of the sugar in solution. Employing a tube 189·4 mm. in length, the percentage of dextrose present in the urine can at once be read off, each degree being equal to 1 gram per 100 c.c. For highly coloured urine a tube 94·7 mm. is used.

Before being examined with the polariscope the urine must be freed from proteins by acidifying 100 c.c. with a few drops of acetic acid, boiling, cooling, filtering, and making up to 100 c.c. again, at the same temperature as the original measurement was made at. The removal of proteins is essential, for each gram of albumen rotates the plane of polarised light equally, but in an opposite direction, to one gram of dextrose.

If the urine is too highly coloured to permit of a satisfactory estimation with either the long or the short tube it may be clarified, but it is necessary to allow for any alteration in volume if this is done. Bone-black has been recommended for the purpose, but as it has been shown to remove a considerable amount of sugar it should not be employed. Kieselguhr is useful to remove turbidity, but it is not always satisfactory, and may also remove some sugar. Neutral acetate of lead is perhaps, on the whole, the most generally useful clearing reagent. It may be used as crystals, or as a 10 per cent. solution, but an excess alters the physical properties of the urine and removes some sugar from solution.

Before the tube is filled with the urine it is carefully cleaned and dried. The cover-glasses should be free from scratches, and also be thoroughly clean and dry. Unnecessary warming by the heat of the hand should be avoided. To fill the tube it is first closed at one end with the cover-glass and screw-cap, then grasped at the other with the thumb and finger, and the urine poured in until the meniscus projects slightly above the opening. Air-bubbles are allowed time to rise, and the second cover-glass pushed horizontally over the end of the tube in such a way that the excess of liquid is carried over the side, leaving the cover-glass exactly closing the tube, without air-bubbles beneath it, and without any liquid on its upper surface. When the cover-glass is in position the tube is closed with the screw-cap, care being taken that too great pressure is not exerted, for this might induce double refraction, and give rotatory power to the glass itself and thus give rise to erroneous readings. The rubber washers must therefore be placed in proper position and the caps be screwed on lightly.

Before taking a reading the zero point of the instrument must be determined, as this changes somewhat with the temperature, &c. The tube is then inserted, the field focused sharply, and the rotation estimated. To find the end-point, with fields of equal illumination, two methods are commonly used. In one the analyser is rotated until a black band seems to cross the division of the fields. This shadow, which is a purely subjective phenomenon, appears a little too soon, but by taking an average of the readings made from both directions, an accurate determination can be made. In the second method the analyser is slowly turned, always in the same direction, the eye being used for only a few seconds at a time, until the end-point seems to be just reached—that is to say, until there seems to be no perceptible difference between the two halves of the field. But since this point is always reached a little too soon, the degree depending upon the

sensitiveness of the instrument, an average of several readings, taken in both directions, must be made to obtain the exact figure. In all cases it should be remembered that the eye must be used for only a few seconds at a time, to prevent fatigue of the retina. The depth of illumination of the whole field, and not of contiguous portions, should be judged. If when the tube is rotated on its long axis the fields change in relative intensity it means either that the cover-glasses are not at right angles to the long axis of the tube, or that they are subjected to too much pressure from the screw-caps. If the whole of the field is not equally sharp the solution is not homogeneous, or the tube is dirty.

Since normal urine is slightly levo-rotatory ($0.05°$ to $0.18°$) a trace of dextrose may produce no noteworthy rotation. Indican, peptones, cholesterin, and the administration of certain alkaloids cause the urine to be levo-rotatory, but indican is usually only present in small amounts in diabetic urines, cholesterin occurs but rarely, and the concurrence of glucose and peptones has not been observed. The most important levo-rotatory substances are levulose and beta-oxybutyric acid. Oxybutyric acid is practically always associated with the presence of dextrose, and may be recognised by fermenting the urine, filtering, and again examining with the polariscope, when it will be found to be distinctly levo-rotatory. Levulose also generally occurs in association with dextrose. They may be estimated together in the following manner :—

Let x represent the dextrose, and y the levulose. If fermentation of the urine shows 2 per cent. of sugar and polarisation 1.2 per cent., then—

$$x + y = 2.0$$
$$x - y = 1.2$$
$$\therefore\ 2x = 3.2$$
$$\text{and } x = 1.6, \text{ and } y = 0.4.$$

If, after complete fermentation, the polariscope still shows 0.3 per cent., owing to the presence of β-oxybutyric acid, then—

$$x + y = 2.0$$
$$x - y = 1.2 + 0.3 = 1.5$$
$$\therefore\ 2x = 3.5$$
$$\text{and } x = 1.75, \text{ and } y = 0.25.$$

Estimation of Levulose.—The reducing actions of levulose and dextrose on *Fehling's and other alkaline copper solutions* are usually assumed to be the same, although Soxhlet states that the reducing power of the former is sensibly less than of the latter, 10 c.c. of Fehling being reduced by 0.0543 gram of levulose as compared with 0.05 gram of dextrose. On account of its ketonic nature levulose reduces alkaline solutions of copper more rapidly, and at a lower temperature, than other sugars, and this property has been utilised for its estimation.

Pieraerts obtained the best results with a cupro-glycocoll solu-

tion, consisting of 6 grams of cupric hydroxide, 12 grams of glyco-
coll, and 50 grams of potassium carbonate, dissolved in water and
made up to 1000 c.c. This solution is reduced by levulose at
normal temperature in twelve hours, but is totally unaffected by
other sugars in twenty-four hours. To carry out the determination,
the urine is treated with basic lead acetate, filtered, and the excess
of lead removed by adding a saturated solution of sodium sulphate.
After half an hour the mixture is again filtered, diluted if necessary
so as to contain 5 per cent. of reducing sugar, and mixed with the
reagent.

The reducing action of levulose on *Knapp's solution* is about
the same as that of dextrose, 1 gram of levulose in 1 per cent.
solution reducing 508·5 c.c. of the reagent ; but Sachsse's solution
is more markedly reduced by levulose than by dextrose, 1 gram
of levulose in 1 per cent. solution reducing 449·5 c.c.

When a urine contains only levulose it is most readily esti-
mated by means of the *polariscope*, the levo-rotation due to the
sugar being distinguished from that arising from other causes
by the former disappearing after complete fermentation. It is
to be noted, however, that the rotatory power of levulose is markedly
influenced by the concentration, and temperature, of the solution,
and particularly by the latter. At 15° C. [a] has a value of $-93·8°$.
This value decreases 0·6385° for each rise of 1° C. in the tempera-
ture, so that at 87·2° C. it is $-52·7°$, which is equal, but opposite
to that of dextrose at the same temperature. This change in
optical activity affords a means of estimating levulose in the
presence of other sugars. The solution is examined in a jacketed
polarimeter tube provided with a thermometer, and the rotation
noted at two temperatures as far apart as possible. Provided that
the solution is fairly strong, the difference between the two tem-
peratures multiplied by 1·277, and the product divided into 100
times the alteration measured in rotation in circular degrees, in a
2 decimetre tube by sodium light, gives the number of grams of
levulose in 100 c.c. As the rotatory power of other sugars is also
affected somewhat by change of temperature, the results are not
strictly accurate.

For *clinical purposes* in urinary analysis it is generally assumed
that the reducing powers of levulose and dextrose are identical,
and when the two are present together in a urine, as is generally
the case, the rotation is usually measured in terms of dextrose.
The percentages of each sugar present is then estimated by com-
paring the readings obtained with the polariscope and by titra-
tion, or fermentation, as in the example already given (p. 99).

Estimation of Maltose.—The reducing power of maltose for hot *Fehling's solution* is only about 62 per cent. of that shown by dextrose, 10 c.c. of Fehling being reduced by 0·0807 grams of maltose, as compared with 0·05 grams of dextrose. The amount of reduction that occurs varies with the concentration of the solutions. Soxhlet states that the cupric reducing power is 61 when the maltose is present in 1 per cent. solution, the Fehling solution is undiluted, and is employed in the exact proportion necessary—64·1 when the copper solution is previously diluted with 4 volumes of water, and 65·3 per cent. when twice as much of the diluted Fehling solution is used as is required for the reaction. If a solution of maltose is treated with a volume of Fehling's solution sufficient for its oxidation, the mixture heated and the cuprous oxide filtered off, a solution is obtained which, if acidulated with hydrochloric acid and heated, acquires the property of reducing an additional quantity of Fehling solution. The second reduction is somewhat more than half the first, so that the two together approach the reducing power of dextrose.

The reducing action of maltose on *Pavy's solution* is the same as upon the ordinary Fehling's solution, but the addition of more sodium hydroxide, in the presence of ammonia, increases the oxidising power of the copper solution to a notable extent.

One gram of maltose in 1 per cent. solution corresponds to 317·5 c.c. of *Knapp's*, and 197·6 c.c. of Sachsse's, solution.

Maltose has a value for $[a]_v = +138°$.

Maltose is not directly fermented by ordinary brewer's yeast, but is first hydrolised to dextrose by the enzyme maltase present. Some species of yeast—*S. marxianus, S. exiguus, S. Ludwigii, W. anomala, W. Saturnus*—do not contain "maltases," and are therefore incapable of fermenting maltose, so that by means of them dextrose and levulose may be detected, and estimated, in the presence of maltose, and by comparing the results obtained by titration and fermentation the percentage of maltose present may be estimated.

Estimation of Lactose.—The reducing power of milk-sugar for *Fehling's* solution is considerably less than that of dextrose, 10 c.c. of Fehling requiring 0·0678 gram of lactose to effect complete reduction, as compared with 0·05 gram of dextrose.

If the reduced copper oxide is filtered off, the filtrate acidulated with hydrochloric acid and heated, hydrolysis of the disaccharide occurs, and a further quantity of Fehling's solution will then be reduced on heating, as in the case of maltose, the second reduction being about half the first.

Lactose is dextro-rotatory, the value of $[a]_D = +52 \cdot 7°$.

Estimation of Pentoses.—Pentoses occurring in the urine cannot be accurately estimated by titration with *Fehling's solution*, as they do not give a sharp end-point. Bial has made use of *Knapp's solution*, and Salkowski estimated the amount of pentose in his case from the quantity of *pentosazone* obtained from a measured volume of the urine. The following method for determining pentoses in urine has been suggested by Jolles as being more accurate.

1. *Jolles' Method.*—The furfurol formed by distilling the pentose-containing urine with hydrochloric acid is estimated by combining it with sodium hydrogen sulphite.

The urine is acidified with a few drops of acetic acid, boiled, and concentrated if necessary, to free it from interfering volatile substances. One hundred cubic centimetres are then mixed with 150 c.c. of hydrochloric acid (sp. gr. $1 \cdot 06$, *i.e.* about 12 per cent. HCl), placed in a flask, and submitted to steam distillation, taking care that the contents of the flask do not fall below about 100 c.c. When the distillate amounts to about 1000 c.c., and a sample no longer gives Bial's orcin reaction, 100 c.c. are neutralised with 20 per cent. sodium hydroxide, using methyl orange as the indicator. A drop or two of half normal hydrochloric acid is then added, so that the red coloration is just restored. A measured volume of standard sodium hydrogen sulphite, sufficient to more than combine with all the furfurol present, is then added and the mixture allowed to stand for two hours. The excess of sulphite is now titrated with $\frac{1}{10}$th normal iodine solution, using starch paste as the indicator. Each cubic centimetre of normal sodium hydrogen sulphite lost corresponds to $0 \cdot 07505$ gram of pentose.

2. *Phloroglucin Method.*—The furfurol formed on distilling a pentose-containing urine with 12 per cent. hydrochloric acid by direct heat may be estimated as a phloroglucinol compound.

A quantity of pure phloroglucinol (see Allen's *Commercial Analysis,* vol. i., 401, 1909), about double the amount of furfurol expected, is dissolved in 12 per cent. hydrochloric acid, and added to the distillate. The mixture is well stirred, and left to stand overnight. The amorphous black precipitate that forms is filtered off through a weighed gooch crucible, provided with an asbestos felt, and carefully washed with water, then dried at 100° C. for four hours, cooled, and weighed. To calculate the furfuroldehyde or pentose present the following formulæ (Kröber) are used, where " a " represents the weights of phloroglucin found.

For weights of phlorogluide under $0 \cdot 03$ grams—

$$\text{Furfurol} = (a + 0 \cdot 0052) \times 0 \cdot 5190$$
$$\text{Pentose} = (a + 0 \cdot 0052) \times 1 \cdot 0170$$

For weights of phloroglucide from 0·03 grams to 0·3 grams.

$$\text{Furfurol} = (a + 0·0052) \times 0·5185$$
$$\text{Pentose} = (a + 0·0052) \times 1·0075$$

For weights of phloroglucide over 0·300 grams—

$$\text{Furfurol} = (a + 0·0052) \times 0·5180$$
$$\text{Pentose} = (a + 0·0052) \times 1·0026.$$

Gründ gives the following formulæ for arabinose and xylose—

$$\text{Arabinose} = \text{phloroglucide} \times 1·148 + 0·0025$$
$$\text{Xylose} = \text{phloroglucide} \times 1·045 + 0·00305$$

Estimation of Glucuronic Acid.—Like the pentoses, glucuronic acid cannot be satisfactorily estimated by the usual titration methods, but it yields furfurol on being distilled with hydrochloric acid, and by combining this with phloroglucin the quantity may be estimated. One part of furfurol-phloroglucide corresponds to three parts of glucuronic anhydride. Other furfurol-yielding substances, and particularly the pentoses, must of course be first excluded.

On being heated with hydrochloric acid, glucuronic acid yields carbon dioxide in addition to furfurol. Lefevre and Tollens have suggested that by weighing the carbon dioxide evolved when its solution is boiled for three or four hours with hydrochloric acid (sp. gr. 1·06), and allowing one part of carbon dioxide for four parts of glucuronic anhydride, the amount of glucuronic acid present in a solution may be determined.[1] The value of this method as applied directly to urine has not yet been proved, but it is probable that the result will be too high, owing to the presence of other substances yielding carbon dioxide.

Since the pentoses do not yield carbon dioxide on being heated with hydrochloric acid, it is possible to estimate the glucuronic acid and the pentose in a sample, when they are present together, the former by the weight of carbon dioxide evolved, and the latter from the quantity of furfurol formed.

Neuberg has employed the para-brom-phenylhydrazin compound of glucuronic acid for its estimation.

Estimation of Sugar in the Blood.—A weighed or measured quantity of blood is freed from albumen by boiling with sodium sulphate, and filtering. The precipitate is well washed, and the quantity of sugar in the filtrate is estimated. A certain amount of sugar is carried down by the precipitate, and unless the blood is quite fresh the action of the glycolytic ferment may cause serious error.

[1] See Abderhalden's *Handbuch de Biochem. Arbeitsmeth.* (1909), ii. p. 139.

For clinical purposes the sugar in the peripheral blood stream may be estimated by collecting 5 c.c. and at once mixing it with 100 c.c. of absolute alcohol, filtering off the precipitate, washing with hot water several times, evaporating the combined filtrates on a water-bath to about 0·5 c.c., and estimating the sugar in this.

The estimation may be carried out by titration with alkaline safranin, or by Lohenstein's saccharimeter. If a large amount of blood is available, titration with Fehling's solution, &c., or polarimetry, may be employed.

Wacker has described a colorimetric method by which it is claimed that sugar can be accurately estimated in such small quantities of fluid as 0·3 to 0·4 c.c., with an error of but 0·01 per cent. The protein of the blood is removed with iron alum and sodium carbonate. To the filtrate phenylhydrazine-p-sulphonic acid and sodium hydroxide are added, and the red colour is compared with a colour scale made with the same reagent and a standard solution of dextrose. The figures given for normal blood by this method are higher than those found by previous observers.

(b) Acetone Bodies

The presence of the secondary disturbances of metabolism that are liable to occur in cases of persistent dextrosuria are shown by the appearance of the acetone bodies (acetone, aceto-acetic acid, and beta-oxybutyric acid) in the urine, and their extent is indicated by the amount of these substances excreted daily. It is therefore most important that their presence should be recognised and that the daily output should be estimated as accurately as possible, both from the point of view of prognosis and treatment.

Chemically the acetone bodies are, as we have seen, intimately related, the oxidation of beta-oxybutyric acid giving rise to aceto-acetic acid and water, and acetone being easily decomposed into acetone and carbon dioxide. It is believed that this series of changes normally takes place in the body. Beta-oxybutyric acid is not met with in health urines, although Gerhardt produced an excretion of about 9 grams in the twenty-four hours by feeding a normal individual on a purely protein diet for some days, and its presence may be regarded as distinct evidence of a perversion of metabolism. Since it is the mother substance of acetone and aceto-acetic acid, the latter are always present in the urine when beta-oxybutyric acid is being excreted. Only a trace, if any, aceto-acetic acid occurs in normal urines, and probably none on a mixed diet. If it is found, it may be taken for granted that acetone will also be there, and generally, but not necessarily, oxybutyric acid

also. Acetone, unlike the two other acetone bodies, appear to be a constant constituent of the urine, although in very small amounts, 0·01 to 0·03 gram in the twenty-four hours, a quantity which is not revealed by the ordinary clinical tests. The excretion of acetone bodies is usually most marked when the output of sugar is high, but there is no constant relation between the two.

(A) These substances may be recognised by the following *qualitative* tests :—

Acetone.—The urine must be as fresh as possible. If only a small amount of acetone is present it may be advisable to distil, and apply the tests to the distillate, but this is rarely necessary if Lange's or Rothera's modifications of the nitro-prusside test is employed. If these are negative, it may be concluded any acetone that may be present is not of pathological significance.

1. *Nitro-Prusside Test.*—A fresh solution of sodium nitro-prusside when added to an alkaline solution of acetone gives a ruby-red colour, which rapidly changes to yellow. If, while the solution is still red, an excess of acetic acid is added, the colour changes to a purple, and later to violet. Creatinin gives with nitro-prusside and an alkali a similar colour reaction, but it more quickly changes to yellow, an alteration which is instantly brought about by the addition of acetic acid, so that the reaction due to acetone can be distinguished from that caused by creatinin by this means. Paracresol gives a reddish-yellow solution, acetic acid a clear rose colour. Aceto-acetic acid gives the same reaction as acetone.

On these facts are based the following tests :—

Legal's.—About 5 c.c. of the urine (or the distillate) are mixed with 5 drops of a freshly prepared, concentrated (10 per cent.), solution of sodium nitro-prusside, and about 1 c.c. of a solution of sodium, or potassium, hydrate (15 per cent.) is added. An excess of glacial acetic acid is then introduced. In the presence of much acetone a purple red colour appears with a large excess of acetone a deep violet, and with traces a rose-violet. According to Bohrisch this test, when directly applied to the urine gives a satisfactory reaction with 0·1 per cent. of acetone.

Le Nobel.—To exclude aldehyde, Le Nobel and Lee advise the use of ammonia, or ammonium carbonate, in place of sodium or potassium hydrate, but the colour change does not take place so rapidly unless a drop of acetic acid is added.

Lange.—As in the preceding method, ammonia is the alkali used, but the delicacy of the reaction is increased by carrying it out as a " ring-test." To 15 c.c. of the urine, contained in a test-tube, are added 0·5 to 1·0 c.c. of glacial acetic acid, and a few drops of a freshly prepared

concentrated solution of sodium nitro-prusside. These are mixed, and then a few cubic centimetres of strong ammonia are run on to the top. In the presence of acetone a violet ring forms at the junction, its depth and intensity varying with the amount of acetone. This test is said to show 0·0025 per cent. of acetone.

Rothera has suggested the following modification of nitro-prusside test : 5 to 10 c.c. of the urine are saturated with solid ammonium sulphate (or according to Garrod, mixed with an equal volume of a saturated solution) by vigorous shaking. Two or three drops of a fresh 5 per cent. solution of sodium nitro-prusside are then introduced, and 1 to 2 c.c. of concentrated ammonia added. The presence of acetone is shown by the formation of a very characteristic " permanganate " colour, which is well seen in the foam on the top of the mixture.

2. *Salicylaldehyde Test (Frommer).*—This is based upon the fact that acetone reacts with salicylaldehyde to form dioxydibenzoylacetone. The test may be applied directly to the urine, and gives no reaction with aceto-acetic acid if the temperature is not raised too high. It is said to indicate the presence of 0·000001 gram of acetone in 8 c.c. of water, and is not affected by the presence of protein.

Ten c.c. of the urine are rendered strongly alkaline with potassium hydroxide, and 10 to 12 drops of a 10 per cent. solution of salicylaldehyde in absolute alcohol are added. The mixture is then warmed to 70° C. In the presence of acetone the fluid becomes yellow, then red, and later purplish-red, turning to dark red on prolonged standing. If acetone is absent the colour of the urine is practically unchanged. It may also be carried out as a ring-test, adding 1 gram of solid sodium hydroxide to about 10 c.c. of the urine, and, without waiting for it to dissolve, running in 10 or 12 drops of the salicylaldehyde solution and warming to 70° C.

Other methods of detecting acetone in the urine have been described by Reynolds (fresh mercuric oxide), Penzoldt (indigo test), Fröhner (hydroxylamine), Vournasos (isonitrile test), and others, but it is not necessary to describe them in detail here, and the reader is referred to the original papers, to which reference is made in the bibliography.

3. *Iodoform Test.*—Like other substances containing an easily oxidised $-CH_3$ group, acetone forms with iodine, in alkaline solutions, iodoform, and by this its presence can be detected. The test cannot be applied directly to the urine, but only to the distillate.

Distillation.—200 to 250 c.c. of the urine are acidified with acetic, or better with phosphoric acid, and distilled with an efficient, well-cooled condenser. The latter precaution is especially necessary for

quantitative work. If the urine is distilled with steam, as v. Jaksch advises, it is not necessary to acidify it. Most of the acetone passes over in the first 10 to 30 c.c., which are used for the tests. If it is desired to exclude aceto-acetic acid, which is seldom necessary, the urine should be previously made alkaline and thoroughly extracted with alcohol free ether. The ether extract may be shaken out with water, and the latter used for the aceto-acetic acid tests. Instead of being distilled the acid urine may, according to Bohrisch, be extracted with ether, 20 c.c. for each 30 to 50 c.c. of the urine, the ether extract be shaken out with water, the residual ether be driven off from the watery extract by warming at 40° C. for about five minutes, and the residue, which contains the greater part of the acetone, can be used for the tests.

Lieben's Iodoform Reaction.—One c.c. of the distillate is mixed with a drop of strong sodium hydrate solution, and sufficient iodo-potassium iodide solution (iodine 1 : pot. iod. 2 : water 100) to give a feeble yellow colour. If no turbidity appears in a few minutes the test-tube is placed in a water-bath at 70° C. for a few minutes, and then allowed to cool. With only 0·01 mg. of acetone a precipitate of iodoform, recognised by its smell and appearance microscopically as yellow six-sided plates or stars, appears in 1 to 3 minutes, and with 0·0001 mg. in 24 hours. The iodoform crystals must be carefully distinguished from the stellate triple phosphate crystals that are also often found. If necessary, the precipitate may be recrystallised from ether. Urines preserved with thymol give a pink, or red, colour due to the formation of di-iodothymol, but no other substance than acetone occurring in the urine yields iodoform.

Gunning's Modification.—To 5 c.c. of the distillate add a few drops (5 to 10) of 10 per cent. ammonia, and about 5 drops of alcoholic iodine solution (tinct. iodi). A deep black precipitate of nitrogen iodide forms. On standing this gradually disappears and leaves a yellow deposit of iodoform, if acetone is present.

The preceding tests that can be applied directly to the urine may also be used for the detection of acetone in the distillate, also Béla's dinitro-benzol test, Rosenthaler's vanillin test, and Reynolds' mercuric oxide test, which only shows over 1 per cent. in the urine direct.

Aceto-acetic (Di-acetic) Acid.—The urine used for testing for this substance must be fresh, for it quickly breaks down, and may not be detected after standing for twenty-four to forty-eight hours.

1. Aceto-acetic acid, and its salts, give with ferric chloride a violet-red colour, which is red-brown with an excess of the reagent. The colour fades on standing for twenty-four hours in the cold, and more rapidly on warming, or adding a mineral acid.

Gerhardt's Ferric Chloride Reaction.—To 10 or 20 c.c. of the urine add a solution of ferric chloride, which must not be too acid, drop by drop,

so long as any precipitate forms, and then filter. To the filtrate is now added a few more drops of ferric chloride, when, if aceto-acetic acid is present, the urine will assume a Bordeaux-red colour, which is cherry-red by transmitted, and purple-red by reflected light. On heating or standing the intensity of the colour diminishes.

A similar colour reaction is also given by other substances that may occur in the urine, such as cyanates, sodium acetate, salicyclic acid, phenol, skatoxyl compounds, and substances excreted after the administration of aspirin, diuretin, salol, antipyrin, thallin, chinisol, &c. In the case of antipyrin, thallin, and chinisol urines the colour does not appear until after the lapse of two or three minutes. With salicyluric acid the colour is violet, with antipyrin and thallin purplered, turning in the latter to red-brown in three or four hours. The colour with all these substances, in contrast to that given by aceto-acetic acid, is stable, and on heating only the colours due to aceto-acetic acid, ferric acetate, and thallin are destroyed. If the test is repeated with a specimen of the urine which has been feebly acidified with sulphuric acid, boiled, and cooled, the red colour should be less marked, as the aceto-acetic acid will have been partly broken down into acetone, but boiling for half an hour will not decompose it entirely.

The aceto-acetic acid may be separated and tested for by acidifying the urine with sulphuric acid, extracting with ether, shaking the ether extract with water, and adding ferric chloride, when a violet-red colour will appear in the watery layer. Salicyluric acid may be removed by shaking the urine with benzol, or chloroform, in which aceto-acetic acid is insoluble.

2. *Arnold-Lipliawski Test*.—This test is said to be more delicate than Gerhardt's, and does not give a confusing reaction with other substances.

Two solutions are required—(1) para-amidoacteophenone 1 gram, concentrated hydrochloric acid 2 c.c., distilled water 100 c.c. ; (2) potassium, or sodium, nitrite 1 gram, distilled water 100 c.c. Six c.c. of the first solution, and three of the second, are mixed together. An equal volume of the urine is then added, a drop of ammonia introduced, and the mixture shaken. A bright red colour results. From a $\frac{1}{2}$ to 2 c.c. of the liquid, the amount depending upon the quantity of aceto-acetic acid present, is then mixed with 10 to 20 times its bulk of concentrated hydrochloric acid (1·19) to which 2 to 4 drops of ferric chloride have been added. From 2 to 3 c.c. of chloroform are then added, and mixed, without shaking. In the presence of aceto-acetic acid the chloroform is coloured violet in $\frac{1}{2}$ to 1 minute. Otherwise it only turns yellow.

3. *Mörner's Test*.—When a urine containing aceto-acetic acid is heated with a little iodide of potassium, and an excess of ferric chloride, it gives rise to the smell of acetone iodide. The reaction

is not specific, however, as urines containing much acetone, but no aceto-acetic acid, also give it (v. Jaksch).

Bondi and Schwartz's Modification.—To 5 c.c. of the urine is added a solution of iodo-potassium iodine, drop by drop, until the fluid is orange-red. It is then decolorised by being gently warmed. A further addition of the iodine solution is made until the urine remains a faint red, even on warming. If the mixture be now gently boiled, the piercing smell of acetone iodide will be noticed if aceto-acetic acid is present. This reaction, while more delicate than Gerhardt's, is said not to be given by acetone, or oxybutyric acid. It is only obtained with neutral, or feebly acid, urines, so that alkaline urines must be previously made neutral with acetic acid.

Riegler's test has been much criticised and its negative nature is a distinct drawback.

One to two c.c. of normal urine are mixed with 2 c.c. of 10 per cent. solution of iodic acid, and 3 c.c. of chloroform, and shaken. The chloroform is thereby coloured violet. If 10 c.c. of the suspected urine are now added, and the mixture shaken, the presence of aceto-acetic acid is shown by the chloroform being decolorised, owing to the acid combining with the free iodine.

Beta-oxybutyric Acid.—This is the mother substance of aceto-acetic acid, and consequently need be only looked for when the urine gives the ferric chloride, or a similar reaction, for aceto-acetic acid. It may not, however, be found even when aceto-acetic acid is present. If some simple test for oxybutyric acid could be devised, it would be of great value in the investigation of cases of glycosuria, but, unfortunately, such does not exist, and the presence of beta-oxybutyric acid can only be demonstrated by investigations too complicated for routine clinical work, or be inferred from indirect evidence.

1. Beta-oxybutyric acid may be suspected if the amount of sugar in a urine, as determined by titration or fermentation, is distinctly larger than with the polariscope, since the acid is levo-rotatory, $[a]_p = -24 \cdot 12°$. Other levo-rotatory bodies, such as albumen, paired glucuronic acid, and levulose, may, however, bring about a similar result, and must first be excluded.

2. It is probably present if the fermented urine of a diabetic is distinctly levo-rotatory after other levo-rotatory substances have been removed by treating it with basic lead acetate and ammonia, and filtering off the precipitate. The levo-rotation may still, however, be due to compound glucuronates, but these may be excluded by strongly acidulating the fermented urine with phosphoric acid, extracting with ether, and examining the ethereal extract with

the polariscope. If this is also levo-rotatory, the presence of oxybutyric acid may be assumed with some confidence.

3. *Conversion into Crotonic Acid.*—On being heated with sulphuric acid, oxybutyric acid is converted into crotonic acid, which separates out as white crystals with a melting-point of 71° to 72° C.

The urine is mixed with an equal quantity of strong sulphuric acid, placed in a flask, provided with a drop-funnel, and distilled. From time to time additions of water are made through the funnel to keep the mixture at a constant bulk. The first part of the distillate is collected in a test-tube and well cooled, when, if considerable amount of crotonic acid is present, it will crystallise out. The crystals are separated, dried between blotting-paper, and their melting-point taken. This should lie between 70° to 72° C. If no crystals appear the distillate is shaken with ether, the ether extract separated, and allowed to evaporate spontaneously. Any crystals that appear are washed with water, dried, and examined for their melting-point. The crystals may be further purified by redissolving them in ether, evaporating, and precipitating the residual fluid with petroleum ether, which separates the traces of fatty acids that also distil over. (Embden and Schmitz.)

4. *Conversion into Aceto-acetic Acid.*—On oxidising beta-oxybutyric acid with hydrogen peroxide and ferrous sulphate, it is converted into aceto-acetic acid, which can be recognised by the ferric chloride reaction.

Black's Method.—5 to 20 c.c. of the urine are evaporated, by gently heating on a water-bath, to $\frac{1}{3}$ to $\frac{1}{4}$ of their volume, thereby expelling the preformed aceto-acetic acid. The residue is acidified with a few drops of concentrated hydrochloric acid, and mixed with recently burned gypsum to a thick paste until it begins to solidify. The mixture is then thoroughly ground and extracted twice with ether, stirring well. The ether is evaporated off, the residue dissolved in water, and neutralised with barium carbonate. Two or three drops of hydrogen peroxide are added to the neutral fluid, and then a few drops of 5 per cent. ferric chloride, that contains a trace of ferrous salt. If the urine contained oxybutyric acid a rose-red colour appears in a few seconds. On standing the colour gradually intensifies for a time, but subsequently slowly fades. The test solution must be cold and neutral in reaction. An excess of hydrogen peroxide and iron must be avoided. According to Black, this test shows 1 : 10000 of oxybutyric acid.

Embden and Schmitz have modified the preceding method by directly testing the acid ether extract of the urine, that has been freed from aceto-acetic acid by heat. To the ethereal extract they add a few drops of hydrogen peroxide, and then cautiously, drop by drop, a 5 per cent. solution of ferrous sulphate. If beta-oxybutyric acid is

present the typical violet colour of the ferric chloride reaction for aceto-acetic acid is produced.

5. *Conversion in Acetone.*—By more prolonged heating the aceto-acetic acid may be converted into acetone, which can be tested for by the nitro-prusside test.

Hart's Method.—Twenty c.c. of the urine are diluted with an equal amount of water, acidified with a few drops of acetic acid, and evaporated down to 10 c.c. to drive off the acetone and aceto-acetic acid originally present. The residue is made up to 20 c.c. again. One c.c. of hydrogen peroxide is added, and the mixture warmed, but not boiled, and then cooled. It is placed in a test-tube, 1 c.c. of glacial acetic acid, and a few drops of a freshly prepared concentrated solution of sodium nitro-prusside added, and the mixture overlaid with strong ammonia. If the urine contained oxybutyric acid a purple-red ring will appear at the junction in 4 to 5 hours, and on shaking the whole of the fluid will become coloured. This test is said to show 0·3 per cent. of beta-oxybutyric acid.

(B) The acetone bodies in the urine may be *estimated* separately or together by direct methods, but, as the processes involved are lengthy and laborious, it is more usual for clinical purposes to infer the extent of the metabolic disturbance which their presence indicates by an indirect method—namely, by a determination of the amount of nitrogen excreted in the twenty-four hours in the form of ammonia.

Ammonia nitrogen.—A small fraction, about one-twentieth as a rule, of the total nitrogen eliminated in the urine is in the form of ammonium compounds, and is usually referred to analytically as ammonia nitrogen. The normal amount of ammonia nitrogen contained in adult urine varies between 0·3 and 1·2 grams in the twenty-four hours, with an average of 0·7 grams (Neubauer). In persistent glycosuria the excretion is often very much increased, occasionally reaching as much as 8 grams in the twenty-four hours, the excess being accounted for by the ammonia combined with aceto-acetic and oxybutyric acids. The amount of ammonia nitrogen eliminated may therefore be used as an index of the quantity of these two substances that is present. Although sufficiently exact for routine clinical purposes, estimation of the ammonia nitrogen only approximately measures the pathological acids, for, as we shall see when we come to consider diabetic coma and "acidosis," ammonia is only used to neutralise these acids when fixed alkalies are no longer available. According to Magnus-Levy, about 1 to 2 grams of the ammonia excreted by a diabetic are used to neutralise the fixed excess of acid resulting from the protein

diet, and it is only the remainder that is the measure of the patho-logical excretion of acid. A diabetic with 4 grams of ammonia in his urine is consequently not excreting twice, but nearly three times, as much pathological acid as one with 2 grams, since 1 gram or more of the 2, or 4, respectively is combined with the acids nor-mally excreted. According to this view, when 2 grams of ammonia are excreted, about 1 gram is combined with oxybutyric and aceto-acetic acids. An output of 5 grams leaves 3 to 4 grams for this purpose, and an excretion of 8 grams gives from 6 to 7 grams of ammonia as the measure of the pathological acids. These figures correspond to 5 grams, 20 grams, and 36 to 40 grams of beta-oxybutyric acid respectively. Magnus-Levy points out that an estimation of the pathological acids from the ammonia excretion may vary considerably as the result of a temporary retention, or excretion, of fixed alkalies, and that an isolated observation may lead to erroneous conclusions. The estimations must therefore be made with the total twenty-four hours' urine, and on several successive days. He considers that 6 to 7 grams of ammonia, corresponding to 36 to 40 grams of beta-oxybutyric acid, is the maximum amount the organism can provide for the neutralisation of pathological acids, although much larger quantities of acid are sometimes excreted.

Estimation of Ammonia Nitrogen.—The urine must be as fresh as possible to prevent the inclusion in the estimation of ammonia formed by the bacterial decomposition of urea. Urines which are alkaline from changes undergone in the bladder, or on standing, cannot be used.

1. *Malfatti-Jager.*—This method depends upon the fact that ammonium salts react with formaldehyde, in neutral solutions, to form hexamethylenetetramine, setting free the acids, which can then be titrated with an alkali. The addition of neutral potassium oxalate has been shown by Jager to give a sharper end-point, when phenolphthyalin is used as the indicator.

Ten c.c. of the urine are diluted with about 50 c.c. of distilled water. Three or four drops of a 1 per cent. alcoholic solution of phenolphthyalin, and a few crystals, or better a small quantity of powdered, neutral potassium oxalates are added. Decinormal sodium hydrate solution is then run in from a burette, and the reading taken when a permanent faint pink colour appears. Three c.c. of a neutral solution of formal-dehyde (prepared by diluting commercial " formalin " with an equal volume of distilled water, and adding just sufficient decinormal soda to neutralise the mixture, using phenolphthyalin as the indicator) are then added, when it will be found that the pink colour will disappear.

A further addition of decinormal soda is then made until the pink colour is just restored, and the reading is again taken. The difference between the first and second readings gives the amount of acid that was combined with ammonia. This multiplied by 0·0014 gives the quantity of ammonia nitrogen in 10 c.c. of the urine in grams. The estimation includes the amino acids and substituted ammonias present in the urine, but as a rule these may be neglected.

2. *Folin.*—Twenty-five c.c. of the urine are placed in an areometer cylinder, about 45 cm. high and 5 cm. in diameter. About 1 gram of dry sodium carbonate, 8 to 10 grams of sodium chloride, and a little petroleum or toluol are added, and the neck is closed with a rubber cork provided with a long tube that passes below the surface of the liquid, and a short one that ends in the space above. The shorter tube is connected with a calcium chloride tube filled with cotton-wool, which in its turn is connected with a gas absorption bottle, containing 20 c.c. of decinormal sulphuric acid, 100 c.c. of distilled water, and a few drops of alizarin red as an indicator. The tube leading from the calcium chloride tube terminates below the surface of the contents of the absorption bottle in a special perforated extremity which brings about good contact of the air that passes through with the acid, and so ensures complete absorption of the contained ammonia. A second tube leading from the absorption bottle is connected with a filter pump, and air is drawn through the apparatus for an hour and a half, or two and a half hours if much ammonia is present, at the rate of 600 to 700 litres per hour. The acid in the absorption bottle is then titrated with decinormal sodium hydrate until a red, not a violet, colour appears, and the number of cubic centimetres of acid neutralised by the ammonia is thus determined. Several estimations may be made at the same time by connecting the sets of apparatus in series. This is probably the most satisfactory method for determining the ammonia content of the urine, but it needs a well-fitted laboratory.

Estimation of Beta-oxybutyric Acid.—1. Beta-oxybutyric acid is oxidised by potassium bichromate and sulphuric acid to acetone, the yield being practically a quantitative one when a solution containing 5 per cent. of sulphuric acid is distilled, with the addition of the necessary quantity of a 0·1 to 0·5 per cent. solution of potassium bichromate.

Shaffer's Method.—25 to 250 c.c. of the urine, according to the amount of oxybutyric acid expected to be present, are mixed, in a 500 c.c. flask, with an excess of basic lead acetate, and 10 c.c. of concentrated ammonia. The contents of the flask are diluted to 500 c.c. with water, well shaken, and filtered through a dry filter-paper. Two hundred c.c. of the filtrate are mixed with 200 c.c. of water, 15 c.c. of concentrated sulphuric acid, and a little talcum powder in a distillation flask provided with a drop-funnel. The mixture is then distilled until 200 to 250 c.c. have passed over. This distillate, " A," contains the

H

preformed acetone, and the acetone derived from the aceto-acetic acid, and may be used for their determination. The residue in the flask is diluted with 400 to 600 of a 0·1 to 0·5 per cent. solution of potassium bichromate, through the funnel, and again distilled. During the distillation the volume of the liquid is kept constant by adding water through the drop-funnel to replace that which distils over. If the liquid in the flask assumes a green colour, showing that the bichromate has been used up, a further supply must be immediately introduced through the funnel. After about 500 c.c. of the distillate, " B," have been collected it is mixed with 20 c.c. of hydrogen peroxide, 3 per cent., and a few cubic centimetres of sodium hydrate solution, and redistilled. Three hundred c.c. of this distillate, " B_2," are collected and mixed with 25, or 50, c.c. of decinormal iodine solution, and 10 c.c. of a 40 per cent. solution of potassium hydroxide. The mixture is well shaken and allowed to stand for five minutes, 10 c.c. of concentrated hydrochloric acid are then added, and the mixture titrated with decinormal sodium thiosulphate solution, using starch paste as the indicator. The amount of iodine absorbed by the acetone present represents the oxybutyric acid that the urine contained. The latter may be calculated by allowing 1·794 mg. of beta-oxybutyric acid for each cubic centimetre of the iodine solution absorbed.

2. *Polariscope.*—Since beta-oxybutyric acid is levo-rotatory, $[a]_D = -24·1°$, it may be estimated by means of the polariscope.

The fermented urine is freed from albumen, and decolorised with lead acetate and ammonia, or mercuric nitrate, and its rotatory power determined. According to Minkowski, a rotation of $-1°$ corresponds to $\frac{100}{20·6}$ per cent., or about 5 per cent., of beta-oxybutyric acid, when the 100 mm. tube is used. As the rotatory power of the uric acid, creatinin, and other normal levo-rotatory constituents of the urine is very slight they may be neglected, especially as in most cases of diabetes the amount present is reduced, owing to the polyuria. The compound glucuronates, if present in excess, will affect the result, and it is advisable to exclude them by testing the fermented urine before proceeding to the examination.

Estimation of Acetone (Folin's Method).—The same apparatus is used as in Folin's method for the estimation of ammonia.

Twenty-five c.c. of the urine, 10 drops of phosphoric acid (10 per cent.), 10 grams of sodium chloride, and a little petroleum or toluol, are placed in the areometer cylinder. Into the absorption bottle are introduced 150 c.c. of water, 10 c.c. of potassium hydroxide solution (40 per cent.), and a measured volume of decinormal iodine solution. The apparatus is then connected up, and a current of air drawn through for twenty to twenty-five minutes. The acetone is carried over into

the alkaline hypo-iodite solution and converted into iodoform. Ten c.c. of concentrated hydrochloric acid are now added to the contents of the absorption flask, and, if the iodine present has been in excess of the amount required to combine with the acetone, the original brown colour will be restored. The excess of iodine is then titrated with decinormal sodium thiosulphate solution, using starch as the indicator. Each cubic centimetre of decinormal iodine that has been absorbed corresponds to 0·96 mg. of acetone.

Estimation of Aceto-Acetic Acid.—1. The combined acetone and aceto-acetic acid may be determined from the " A " distillate obtained in Shaffer's method for oxybutyric acid, by making it faintly alkaline, redistilling, and estimating the total acetone by the preceding method. The difference between the preformed acetone, as obtained by Folin's method, and this result will represent the acetone derived from aceto-acetic acid.

2. *Hart's Modification of Folin's Method.*—The same apparatus, and reagents, are used as in Folin's method for estimating acetone.

After the preformed acetone has been removed, the urine is heated nearly to boiling by immersing the areometer cylinder in a water-bath, another absorption flask, charged with a fresh supply of decinormal iodine, being attached. A current of air is drawn through for twenty-five minutes, during which time all the aceto-acetic acid is converted into acetone and carried over into the alkaline hypo-iodite solution. The same procedure is then followed as for the estimation of acetone, the quantity found representing the aceto-acetic acid of the urine.

Numerous other methods for the estimation of oxybutyric acid, aceto-acetic acid, and acetone in the urine have been described, but they are all very complicated and give no more reliable results than the preceding, which are comparatively simple, and are carried out with similar apparatus and reagents.

(c) *Total Nitrogen*

A healthy man, on an average mixed diet, passes in his urine in twenty-four hours from 10 to 16 grams of nitrogen, which, with a daily total of 1·5 litres, works out at 0·67 to 1·07 per cent. The nitrogen-containing constituents of the urine are degradation products of albumen, and their amount is therefore dependent upon the body weight of the individual, the rate of protein metabolism, and the amount of albuminous food material absorbed from the intestine. As we shall see later, an estimation of the total nitrogen of the urine is of importance in persistent glycosuria, both in making a prognosis and estimating the progress of the case, since it indicates the state of protein metabolism within the organism, and also

gives data from which the exact degree of the difficulty the patient has in dealing with carbohydrates can be calculated.

Estimations of total nitrogen in the urine are now almost exclusively made by Kjeldahl's process, or some modification of it, for no other gives such consistently reliable results. The principle of the method is, that the organic constituents of the urine are destroyed by oxidation when it is heated with concentrated sulphuric acid, the nitrogen of those substances which do not contain it combined with oxygen, forming ammonium sulphate. The urea is converted into ammonia and carbon dioxide. On treating the acid solution with sodium or potassium hydroxide, and distilling, the ammonia passes over, and may be estimated by collecting it in a measured volume of an acid solution of known strength. From this the nitrogen may be easily calculated.

1. *Gunning's Modification of Kjeldahl's Process.*—Five c.c. of the clear filtered urine are accurately measured, with a pipette, into a Kjeldahl's digestion flask of Jena glass of about 250 c.c. capacity. Ten c.c. of concentrated sulphuric acid (sp. gr. 1·84), and 0·2 to 0·5 gram of crystallised copper sulphate are added. The flask is loosely closed with a glass ball, and placed in a slightly inclined position on a wire gauze in a fume-chamber. It is then gently heated, with a low flame, until frothing ceases and the black material begins to wash down the sides of the flask. The contents of the flask are allowed to cool somewhat, and 5 to 10 grams of potassium sulphate are introduced. The temperature is now gradually raised until the acid boils briskly, and the boiling is continued until the solution becomes clear and has only a pale blue, or green, colour. It is then allowed to cool. When it is quite cold, about 100 c.c. of ammonia-free distilled water are cautiously added, and the mixture transferred, by means of a small funnel to a round-bottomed distillation flask of about a litre capacity. The glass stopper, the digestion flask, and the funnel are rinsed several times with distilled water, and the washings added to the contents of the distillation flask, which are made up to 350 to 400 c.c. About 15 grams of talc, or a few pieces of granulated zinc, and a few drops of an alcoholic solution (1 per cent.) of phenolphthalein are added. The neck of the flask is now closed with a stopper provided with a tapped funnel, and a Reitmair or Hopkins bulb-tube, which is attached to an efficient condenser. The delivery tube from the condenser is connected with a glass tube, provided with a bulb about half-way along its length, which dips to the bottom of a carefully measured quantity (50 c.c.) of decinormal hydrochloric, or sulphuric, acid contained in an Erlenmeyer flask of about 250 c.c. capacity. The contents of the distillation flask are now made distinctly alkaline by adding about 80 c.c. of sodium hydrate solution (sp. gr. 1·230, or 250 grams per litre) through the funnel, and mixed by a gentle rotatory motion. They are

then distilled. When 100 to 150 c.c. have distilled over, and the steam no longer gives fumes of ammonium chloride when a glass rod moistened with hydrochloric acid is held in front of the open end of the delivery tube, the contents of the receiver are titrated with decinormal sodium hydrate solution, using cochineal, methyl orange, or congo-red as the indicator. The number of cubic centimetres of decinormal sodium hydroxide used is deducted from the number of cubic centimetres of decinormal acid placed in the receiver, and the difference multiplied by 1·404. The result gives the amount of nitrogen contained in the quantity of urine taken, in milligrams.

2. At best the distillation process is a lengthy one, so to save time, and also to avoid the error introduced by the action of the hot alkali on the glass, the ammonia formed can be directly estimated in the neutralised oxidation product by the Malfatti formalin process, as described for ammonia nitrogen.

Rona and Ottenberg give the following directions : 5 c.c. of the urine are mixed with 10 c.c. of concentrated sulphuric acid, and 5 to 8 drops of a 1 per cent. solution of platinum chloride, and heated in a Kjeldahl flask. The digested product is transferred to a 350 c.c. Erlenmeyer flask and made up to 100 c.c. with distilled water, 6 or 7 drops of neutral litmus solution (Kübel-Tiemann), and 20 c.c. of a 30 per cent. solution of sodium hydrate, are then added, and the flask is well cooled in running water. When it is quite cold, 30 per cent. sodium hydrate is gradually added, at first a cubic centimetre at a time, and later drop by drop, until the fluid is blue, taking care to keep it as cool as possible all the time. It is next feebly acidified with fifth normal acid, and then very carefully neutralised with fifth normal sodium hydrate and fifth normal acid, comparing the colour with that of a solution consisting of 150 c.c. of distilled water, 1 c.c. of fifth normal sodium hydrate, and 1 drop of litmus. To the neutral solution is now added 30 c.c. of neutral formal-dehyde, and 1 c.c. of a $\frac{1}{2}$ per cent. solution of phenolphthalein. The mixture is titrated with fifth normal caustic soda until a violet colour, due to the combined effect of the litmus with phenolphthalein, appears. The number of cubic centimetres of soda solution used gives the ammonia nitrogen content. After a little practice the titration process takes about ten minutes to carry out.

BIBLIOGRAPHY

Arnold, *Zentralb. f. inn. Med.*, 1900 ; *Weiner klin. Woch.*, 1899.
Bang, *Biochem. Zeit.*, 1906.
Béla, *Ann. d. Chem.*, 1892.
Benedict, *Journ. Amer. Med. Assoc.*, 1911.
Black, *Journ. of Biolog. Chem.*, 1908–9.
Bohrisch, *Pharm. Zentralhalle*, 1907.

Bondi and Schwarz, *Wiener klin. Woch.*, 1906.

Byrac, *Bull. d. l. Soc. Chim.*, 1894.

Citron, *Deut. med. Woch.*, 1909.

Einhorn, *New York Med. Record*, 1887.

Embden and Schmitz, Abderhalden's *Handb. d. Biochem. Arbeitmeth.*, ii., 926–7, 1910.

Fehling, *Arch. f. physiol. Heilk.*, 1848 ; *Ann. d. Chem. u. Pharm.*, 1849.

Folin, *Zeit. f. physiol Chem.*, 1902–3.

Fröhner, *Deut. med. Woch.*, 1901.

Frommer, *Berl. klin. Woch.*, 1905.

Gerhardt, *Wiener med. Presse*, 1865.

Gerhardt and Schleisinger, *Arch. f. exp. Path. u. Pharm.*, 1898.

Gerrard, *Chem. Zentralb.*, 1896 ; Allen's *Chem. of the Urine*, 1895.

Gunnung, *Journ. d. pharm. et d. Chim.*, 1881 ; *Zeit. anal. Chem.*, xxviii.

Hart, *Amer. Journ. of Med. Sci.*, 1909.

Hasselbalch and Lindhard, *Biochem. Zeit.*, 1910.

Henninger, *Compt. rend. d. l. Soc. d. Biol.*, 1884.

Hopkins, *Journ. Amer. Chem. Soc.*, 1896.

De Jager, *Zeit. f. phys. Chem.*, 1910.

Jolles, *Zeit. anal. Chem.*, 1907.

Kjeldahl, *Zeit. anal. Chem.*, xxii.

Klimmer, *Zeit. f. Tiermed. N.F.*, 1898.

Knapp, *Ann. d. Chem. u. Pharm.*, cliv.

Lange, *Münch. med. Woch.*, 1906.

Lefevre and Tollens, *Ber. d. deut. Chem. Ges.*, 1907.

Legal, *Breslauer arz. Zeitschr.*, 1883.

Lehmann, *Arch. f. Hygiene*, 1898.

Lieben, *Ann. d. Chem. u. Pharm. Suppl.*, 1870.

Ling, Rendle, and Jones, *Analyst*, 1905 and 1908.

Liphawsky, *Deut. med. Woch.*, 1901.

Lohnstein, *Münch. med. Woch.*, 1899 ; *Allg. med. Zentralzeit.*, 1906.

Malfatti, *Zeit. f. analytic. Chem.*, 1908.

Mörner, *Skand. Archiv.*, 1895.

Neubauer, *Journ. f. prakt. Chem.*, lxiv.

Neuberg, *Ber. d. deut. Chem. Gesch.*, 1899.

Le Nobel, *Arch. d. exp. Path.*, 1884.

Oerum, *Zeit. anal. Chem.*, 1904.

Pavy, *Journ. Chem. Soc.*, 1880 ; *Lancet*, 1884 ; *Chem. Zentralb.*, 1879.

Penzoldt, *Arch. f. klin. Med.*, 1883.

Pflüger and Allihn, *Pflüger's Arch.*, 1898.

Pieraerts, *Bull. Assoc. Chim. Sucr. et Dist.*, 1908.

Reitmair, *Zeit. f. analyt. Chem.*, 1886.

Repiton, *Chem. Zentralb.*, 1907.

Reynolds, *Proc. Roy. Soc.*, 1871.

Riegler, *Münch. med. Woch.*, 1906.

Roberts, *Edin. Med. Journ.*, 1861 ; *Lancet*, 1862.
Rona and Ottenberg, *Biochem. Zeit.*, 1910.
Rosenthaler, *Zentralb. f. analyt. Chem.*, 1905.
Rothera, *Journ. of Physiol.*, 1908.
Sachsse, *Chem. Zentralb.*, 1877.
Sahl, *Deut. med. Woch.*, 1905.
Shaffer, *Journ. of Biol. Chem.*, 1908.
Soxhlet, *Journ. prakt. Chem. N. F.*, 1880.
Udransky, *Zeit. f. phys. Chem.*, 1892.
Vournasos, *Bull. Soc. Chim. d. Paris*, 1904.
Wacker, *Zeit. f. physiol. Chem.*, 1910.

CHAPTER IV

I. *Puncture Diabetes*

In the year 1849 Claude Bernard discovered that if the floor of the fourth ventricle of the brain, between the roots of origin of the eighth and tenth pairs of nerves, is punctured, sugar will shortly afterwards appear in the urine. The glycosuria begins about an hour after the puncture has been made, and lasts only as long as glycogen remains in the liver. If the animal be killed, and the liver examined after the sugar has disappeared from the urine, no glycogen, or at most a trace, is found. Puncture of the ventricle in an animal that has previously been starved for several days gives rise to no glycosuria, and tying the hepatic vessels also prevents the excretion of sugar in the urine.

During the time that the glycosuria exists the proportion of sugar in the blood is considerably increased, and it is from the escape of this excess through the kidneys that the sugar in the urine arises. According to Bernard's theory the hyperglycæmia, and consequently the glycosuria, is due to hyperglycogenesis, but if Pavy's view is accepted it must be assumed that they arise from conversion of the liver glycogen into sugar, instead of into fat and proteid. According to many physiologists the balance of available evidence suggests that normally there is a constant tendency for the liver to convert glycogen into sugar within its cells, through the action of a diastatic, or glycogenolytic, ferment, but that this tendency is held in check by an inhibitory mechanism controlled from a centre in the floor of the fourth ventricle.

This diabetic, or glycogenic, centre appears to exist in all animals, and in man glycosuria has been observed to be associated with the presence of tumours, and other lesions, involving this part of the brain. That puncture of the medulla acts by producing irritation of the centre, and not through its destruction, is proved by the fact that puncture in an anæsthetised animal does not cause sugar to appear in the urine, by the short duration of the glycosuria, and more particularly by the exactly similar effects induced by afferent stimuli reaching the centre through the vagus and other

nerves. Cutting the vagus in the neck causes a transitory glycosuria, and subsequent stimulation of the cut central end causes sugar to again appear in the urine. Stimulation of the central end of the cut sciatic nerve, or irritation of the cardiac depressor nerve, acts in a similar way. Tumours pressing on the vagus nerve, and severe neuritis of the sciatic, or facial, nerves, in man have been observed to be associated with glycosuria.

It is generally stated that the centre exercises its control over the liver through efferent impulses travelling along the spinal cord as far as the upper thoracic region, thence by the upper thoracic spinal roots and rami communicantes into the lower cervical and upper thoracic sympathetic ganglia, from which they pass by the splanchnic nerves to the liver. This conclusion has been arrived at by a study of the effects produced by dividing various parts of these nerve-tracts in animals with a diabetic puncture, but, as MacLeod points out, the procedure involved in all the experiments in which it was found that glycosuria did not follow puncture, coincidentally establish a condition of extreme splanchnic vasodilatation, and a consequent fall of blood pressure, which alone is sufficient to cause the glycosuria produced by vagus stimulation to disappear, or become very much less marked.

How the nervous impulses cause the discharge of sugar is not known with certainty. It is possible that the splanchnic nerves contain secretory fibres which control ferment production by the hepatic cells, as appears to be the case with nerves supplying other glands ; but against this is the fact that atropin, which paralyses all true secretory nerve-endings, does not prevent puncture diabetes. Bernard thought that the increased sugar production was due merely to vasodilatation, but stimulation of the central end of the cut vagus, which is accompanied by a rise of blood pressure and irritation of the cardiac depressor nerve, associated with a fall in the abdominal blood pressure, both give rise to glycosuria. It has also been suggested that the primary effect of the stimulus is exerted upon the pancreas, and that the changes that ensue in the liver are secondary to this. The most recently advanced view is that the excessive output of sugar by the liver in diabetic puncture is dependent upon an increased flow of the secretion of the supra-renals, which are caused to function more actively as a result of the nerve stimulation. By the ingenious method of stimulating the splanchnic nerves after all the abdominal viscera, except the adrenals, had been removed, Asher has shown that these nerves control the secretory functions of the glands. If, therefore, the splanchnic system is abnormally stimulated, a rise of blood

pressure and other symptoms, including glycosuria, will result. It has also been found that if the left supra-renal is cut off from the left sympathetic nerve, no sugar appears in the urine after the medulla has been punctured, and it is therefore thought that the stimulus is transmitted by the left sympathetic to the left supra-renal, whence it passes to the right by the connecting nerves, and as a result the supra-renals are aroused to abnormal functional activity.

In man glycosuria may result from injuries to the head, shock to the central nervous system, and even strong psychical excitement, presumably, at any rate in some instances, through irritation, or stimulation, of the glycogenic centre. In many nervous diseases more or less transient glycosuria may occur, and an existing glycosuria may be increased by intercurrent nervous excitement or irritation.

II. *Drug Glycosuria*

(a) **Phloridzin Glycosuria.**—This is the best known, and most thoroughly studied, form of glycosuria produced by the action of drugs, and offers many points of particular interest. It was first described in a series of papers published by von Mering between the years 1886 and 1889. Phloridzin is a glucoside obtained from the bark of apple, pear, and cherry trees, which, by boiling with dilute acids, is split into dextrose (38·1 per cent.) and phloretin. When given in doses of about 1 gram per kilo by the mouth, or subcutaneously in doses of about 0·3 to 0·5 grams per kilo, to dogs or other animals, it causes a marked but transient glycosuria, which passes off in a few hours, unless the drug is readministered. In well-fed animals as much as 19·1 per cent. of sugar may be present in the urine. During starvation the sugar excretion is less (0·3 to 2·5 per cent.), but is more constant. The amount of sugar excreted does not depend, within wide limits, upon the dose of phloridzin used, if enough is given to produce the maximum effect, so that the sugar in the phloridzin itself cannot be an important factor in the sugar excretion. It is to the action of the phloretin that the glycosuric effect must be ascribed. A single dose of phloridzin does not appreciably affect the amount of glycogen in the liver and muscles, but after repeated doses the glycogen may disappear to a large extent from the liver, and also from the muscles, although the reduction is not as complete as in some other forms of experimental glycosuria. The most remarkable difference between phloridzin and other glycosurias is, however, the absence of a hyperglycæmia, such as might be expected by analogy with the effects produced

by a diabetic puncture, and other conditions, causing the appearance of sugar in the urine. It has been stated that a decreased amount of sugar in the blood (hypo-glycæmia) is, in fact, a characteristic feature of phloridzin diabetes, but the experiments of Pavy, and later of Coolen and Kolisch, have shown that the sugar content of the blood is if anything slightly increased, but never sufficiently to cause marked hyperglycæmia.

Apparently, therefore, phloridzin does not act upon the glycogenic functions of the liver and other organs, but simply causes a draining away of sugar into the urine through the blood, to replace which the stored glycogen is converted into sugar. In confirmation of this view is the fact that, while in other forms of experimental glycosuria ligature of the ureters, or excision of the kidneys, causes a marked rise in the percentage of sugar in the blood, a similar rise does not occur under the same conditions in phloridzin diabetes. The absence of hyperglycæmia, and the relatively slight disappearance of glycogen, in phloridzin glycosuria led von Mering to suggest that the drug has a direct action on the kidneys and makes them more permeable to dextrose, so that the normal sugar of the blood is drained off. Later, von Mering suggested that the phloridzin is taken up by the renal cells and decomposed by them into dextrose and phloretin, the dextrose being excreted in the urine and the phloretin passing back into the blood. There it combines with dextrose to form phloridzin again, and this, in its turn, is decomposed by the renal cells into sugar and phloretin. Owing to a gradual escape of phloretin into the urine the action of the drug slowly diminishes, and a renewed dose has to be given to keep up the glycosuria. The renal character of phloridzin diabetes was first demonstrated by Zuntz, who placed cannulæ in the upper portions of the two ureters, and injected phloridzin into the renal artery of one side. On the injected side sugar appeared in the urine in two minutes, but it was three minutes later before sugar could be detected in the urine coming from the other side, the delay being due to the lapse of time necessary for the transportation of the phloridzin from the injected to the other kidney.

It would seem, however, that the excretion of sugar does not depend upon a condition of the kidneys which simply allows it to leak away, but rather upon actual secretory activity, for disease or injury of the renal tissue diminishes or prevents the glycosuria. Biel and Kolisch have also shown that when phloridzin is passed through the vessels of an isolated kidney, sugar is found in the urine. Phloridzin itself causes necrotic changes in the renal

epithelium after prolonged administration, and diuretics, although they increase the volume of urine, do not raise the excretion of sugar as they do in diabetes with hyperglycæmia. Pavy, Brodie, and Siau have suggested that the sugar that appears in the urine in phloridzin glycosuria is formed in the kidney out of a precursor contained in the blood, and is not the blood sugar. According to their theory the precursor is the sugar that is loosely combined with the serum proteid. This is split off in the kidneys and excreted, the proteid returning into the circulation and there combining with more sugar. When, however, the animal is starved so that no fresh sugar is available, the renal cells attack the proteid molecule itself, setting free the sugar that can be derived from it, and also the nitrogen-containing moiety. Analyses of the urine of animals to which phloridzin has been repeatedly given, and that are then starved, have shown that the elimination of sugar does not cease, but continues at a lower level, while at the same time the elimination of nitrogen is increased until a constant maximum ratio of dextrose to nitrogen (D : N) of 2·8 : 1, that is strikingly similar in different animals, is established. Stiles and Lusk, however, obtained a higher ratio of 3·65 : 1 in dogs with normal kidneys after subcutaneous injections of phloridzin, which corresponds to the ratio found by Mandel and Lusk in human diabetes when the patient is given a diet of meat and fat. Reilley, Nolan, and Lusk have shown that in fasting phloridzinised dogs the D : N ratio does not vary after the ingestion of sufficient meat to double the quantity of nitrogen in the urine, for the sugar is at the same time doubled. The sugar production is therefore proportional to the proteid metabolism, and apparently must be derived from the proteid. That an excessive breaking down of proteids occurs in advanced poisoning by phloridzin during starvation is also shown by the presence of β-oxybutyric acid, aceto-acetic acid, &c., in the urine.

Phloridzin glycosuria is readily produced in man as well as in animals, and the use of phloridzin has been suggested as a test of the functional activity of the kidneys. Von Mering quotes a case in which he gave 1 gram of phloridzin night and morning for a month, with the result that the patient passed from $2\frac{1}{2}$ to 3 litres of urine a day containing 2·7 to 3·7 per cent. of sugar, with a total excretion of 2 kilos 728 grams of sugar for the thirty days. Achard and Delamare, after injecting 15 mg. of phloridzin subcutaneously, found that sugar appeared in the urine in half an hour, and that the glycosuria persisted for three and a half hours. Fifty mg. injected into the same patient produced a

glycosuria lasting six hours, and the quantity of sugar excreted was 14 grams.

(b) **Glycosuria due to Drugs and other Toxic Influences.** —Besides phloridzin, a number of other substances, when taken by the mouth, or injected subcutaneously, &c., may cause a more or less marked glycosuria. Some of these probably do so by stimulating the glycogenic centre in the floor of the fourth ventricle ; others appear to act by stimulating the splanchnic sympathetics, while others again seem to exert a direct influence upon the cells of the liver and pancreas, or upon the renal epithelium.

Bock and Hoffman in 1871 made the interesting observation that the intravenous injection of a slightly hyper-tonic solution of sodium chloride into rabbits gives rise to a glycosuria, which can be prevented by the addition of a soluble salt of calcium to the solution. That the glycosuria in this instance is of central origin,is suggested by the fact that it does not occur if the splanchnic nerves are cut before the injection is made. If, however, the salt solution is injected into the central end of the axillary artery, so that the salt may reach the medulla by way of the vertebral, glycosuria is produced, and is then not prevented by the simultaneous injection of calcium chloride (Fischer).

It has been repeatedly noticed that the administration of morphia may cause hyperglycæmia, and glycosuria. The well-known affinity of this drug for the nervous system suggests that it acts upon the glycogenic centre, but it is possible that, like ether and other asphyxiating substances, it may stimulate the splanchnic sympathetics. It has also been suggested that it exerts a direct effect upon the liver cells (Lépine). In this connection the question of asphyxial glycosuria may be considered, for it has been shown by Underhill that the hyperglycæmia and glycosuria produced by certain drugs, such as morphine, nicotine, pyridin, anæsthetics, &c., can be prevented by the free administration of oxygen.

Asphyxial glycosuria is most easily induced experimentally by clamping the trachea, or injecting curare. After section of all the hepatic branches of the cœliac plexus, glycosuria is no longer produced by clamping the trachea, suggesting that the condition is due to asphyxial stimulation of the nerve centres. It has been found, however, that hyperglycæmia does occur after such isolation of the liver from the nervous system, when the asphyxia is effected by injecting curare, indicating that the profound venosity of the blood, beside acting on the nervous centres, may also directly influence the liver cells. Further experiment has suggested that

the latter action is probably due to the carbon dioxide contained in the intensely venous blood (Macleod), and it is probable that the free administration of oxygen in certain drug glycosurias prevents the hyperglycæmia and appearance of sugar in the urine by removing the excess of carbon dioxide (*e.g.* curare, morphine, amyl nitrite, general anæsthetics).

Glycosuria may follow the administration of caffein, theobromine, and diuretin ; and as the chief effect of these drugs is to produce polyuria, it has been suggested that the glycosuria is a result of their action on the kidneys. The experiments of Nishi have shown, however, that, with diuretin at least, the excretion of sugar is dependent upon impulses transmitted through the sympathetic nerves to the supra-renals. Uranium salts (Chittenden and Lambert), chromic acid and chromates (Pal), and cantharides (Richter) are supposed to produce glycosuria by their action on the renal epithelium.

Other substances, among which may be mentioned alcohol and the toxines of acute infectious diseases, appear to induce glycosuria, chiefly through their action upon the pancreas.

The administration of strychnine, phosphorus, arsenic, mercury, hydrocyanic acid and cyanides, chloral, nitro-benzol, chloroform, acetone, ether, amyl nitrite, and organic or mineral acids, the inhalation of carbon monoxide, extensive hæmorrhages, immersion in water, &c., are said to produce glycosuria. With some there can be no doubt that sugar does appear in the urine, and the explanation is one of those given above ; but with others the mechanism of its production is uncertain, and with others, again, it is probable that glucuronic acid, and not dextrose, is the substance that gives the urine its reducing power. In any case the condition is a slight and transitory one not likely to cause any practical difficulty.

III. *Glandular Glycosuria*

Since the pioneer work of Baumann on the thyroid gland, in 1895, which resulted in the isolation of iodothyrin, physiologists have learnt to appreciate the far-reaching influence of the exchange of matter that is continually going on between the living cells of the organism, and to realise that every tissue of the body is continually forming intermediate and end-products that serve as stimulants for other tissues, or in some way exercise an influence upon the metabolic processes of other organs. It is now believed that chemical correlation, and chemical control, exists between practically every gland and tissue of the body. In some instances

it has been possible to isolate the specific chemical messenger, or
" hormone," through which one tissue activates another.   Thus
Bayliss and Starling have shown that the hydrochloric acid secreted
by the stomach acts upon the mucous membrane of the upper
part of the intestine, and gives rise to a substance to which they
have given the name " secretin " ; this being absorbed into the
blood travels to the pancreas, arousing it to functional activity
and causing a flow of its secretion.   Again, we find the intestine
secreting a specific substance, " entero-kinase," which, reacting
with the trypsinogen of the pancreatic juice, converts it into the
active proteolytic ferment trypsin.   These and similar observations
tend to emphasise the part that specific chemical products play in
regulating the metabolism of the body.

The ductless glands are believed to be particularly important
in this connection, for they appear to be engaged in the elaboration
of internal secretions, the active substances of which are of special
importance in the metabolism of the organism.   Their histological
characters, the absence of a duct system, and their intimate con-
nection with the circulation and lymphatics, suggest that, on the
one hand, they receive material from the blood and lymph, and,
on the other, return specific chemical products to the circulation.
Moreover, it has been shown, as the result of their experimental
ablation in animals, that their internal secretions are necessary for
the normal activity of the nervous system, for the circulation, and
for the healthy metabolism and growth of the organism.   If these
glands cease to function, the loss of their specific products leads
to disease, and possibly to death, while an increase in their activities
may give rise to equally disastrous results.   The many-sided rela-
tions, and inter-relations, existing between them and the tissues
generally render it difficult to clearly demonstrate the particular
part that any one gland plays in the economy, so that progress
has been slow, and the explanations given by different authors of
the phenomena observed on removing a gland are often at variance.
Still, sufficient evidence has now been accumulated to render
possible a provisional theory of the results, and to provide material
for a working hypothesis as to their action upon each other, and the
organism generally.

Among their other functions the ductless glands exert a pro-
found influence upon the carbohydrate metabolism of the body,
and although for many years interest has been chiefly centred in
the pancreas, there can be no doubt that the supra-renals, the
pituitary gland, and the thyroid also play a part which is probably
no less important.   We shall first consider the experimental facts

connecting each of these glands with carbohydrate metabolism, and subsequently deal with the theories that have been advanced with a view to unifying the results.

(*a*) **The Pancreas.**—The anatomical similarity of the pancreas to the salivary glands led the early observers to consider that their functions were also of the same nature, and it was not until C. Bernard discovered, in 1849, that the pancreatic juice was concerned in the digestion of fats, and, in 1856, that it was also capable of acting on proteid materials, that the vastly greater importance of the pancreas, even as a digestive organ, came to be recognised. The part that it plays in the internal metabolism of carbohydrates does not appear to have been suspected until 1875, when Bouchardat suggested that lesions of the pancreas were capable of causing diabetes. The subsequent failure of experimental attempts to produce glycosuria in animals through damming back the pancreatic secretion, by ligature of the ducts, or by conveying the secretion outside the body by a fistula, led such an authority as Cohnheim to deny the connection, and to regard all pancreatic changes met with in diabetes as secondary, or accidental complications. In 1889 von Mering and Minkowski placed the pancreatic theory of diabetes on a secure footing, by showing that total extirpation of the pancreas in dogs is followed by severe diabetes, which persists until the death of the animal. In a series of papers published between 1890 and 1893 these observers, and Minkowski alone, gave further details of the operation, and recorded their experience of its effect on a large number, and great variety, of animals.

After complete extirpation of the pancreas in dogs sugar usually appears in the urine the day after the operation, but occasionally in from three to five hours, and gradually increases in amount until it reaches a maximum of 8 to 10 per cent. about the third day. On a diet of bread and meat, a dog of 8 kilos will then be found to be passing from 70 to 80 grams of sugar in the twenty-four hours. If no food is given, a gradual fall occurs after the third day, and continues until a constant level is attained, but even after seven days' starvation glycosuria is still present. There is a marked increase in the quantity of urine passed, a dog of 7 kilos voiding from 1000 to 1200 c.c. in the twenty-four hours. Although a depancreatised animal eats and drinks well, it rapidly wastes and loses strength, so that death takes place from inanition in about four weeks, even when lung disease, or troubles arising from the invariable disinclination of the operation wound to heal, do not bring about a fatal issue at an earlier date. When the animal is

too weak to move about, the excretion of sugar begins to diminish, although food is being taken, and a few days before death it may disappear altogether, especially when there is suppurative peritonitis. Coincidentally with the fall in sugar excretion acetone, aceto-acetic acid, and β-oxybutyric acid make their appearance in the urine.

Examination of the blood of animals with pancreatic glycosuria shows that it contains a proportion of sugar much in excess of the normal 0·1 per cent., sometimes as much as 0·4 per cent. being found, and when the ureters are tied, or the kidneys are removed, the proportion is still further increased, thus pointing to an accumulation of sugar in the blood as the immediate cause of the glycosuria. Post-mortem the subcutaneous and tissue fat are found to have disappeared, and the muscles are diminished in weight. The most striking phenomenon is a relative hypertrophy of the liver, due apparently to fatty infiltration. In one instance, quoted by Carnot, the liver was found to constitute 8·37 per cent. of the total weight of the body, and to yield 47·5 per cent. of fat. If the animal is killed a few days after the pancreas has been removed. the glycogen normally present in the liver and muscles is seen to have disappeared, or only to be present in traces, no matter whether the animal has been starved or liberally fed. It is usually found, however, in excessive quantities in some situations, such as the epithelium of Henle's tubes, the heart muscle, and the leucocytes, where it does not normally occur in abundance.

Minkowski observed that more sugar is excreted at the height of the glycosuria by a dog that has been well fed previous to the operation, than by one that has had little food. It would therefore seem that the increased proportion of sugar in the blood, and that excreted in the urine in the first instance, comes from the glycogen stored in the liver and muscles from the food previously taken, and that excision of the pancreas has in some way interfered with the power of these tissues to replenish their stock. The source of the sugar when all the available glycogen has been used, and the sugar excretion has fallen to a constant level, appears to be the proteins of the tissues. That this is so is suggested by the results of observations on the dextrose to nitrogen ratio $(D : N)$ in the urine. If this is determined in a fasting depancreatised dog, it is found to have a constant value, which, according to Minkowski, who investigated the urines of seven animals on twenty-two different days, averages about 2·8 : 1. This relation of 2·8 grams of dextrose to 1 gram of nitrogen remains practically unchanged, no matter whether the animal is fasting, or is fed on

I

flesh alone, or on other forms of protein food (*e.g.* plasmon, nutrose, casein, &c.), indicating that the sugar and the nitrogen are derived from a common source, the proteins of the tissues and food. The D : N ratio 2·8 : 1 is, however, not what ought theoretically to be obtained if all the carbon of the proteins were converted into dextrose, for we should then have a ratio of about 7 : 1. It is therefore apparent that only some 45 per cent. of the possible sugar is excreted, which must mean, either that all the carbon of the protein is not converted into sugar, or, that some of the sugar formed is destroyed. The former appears to be the more probable explanation, although many observers maintain that depancreatised dogs still retain slight sugar-destroying powers.

The administration of a moderate amount of dextrose to a depancreatised dog results in the whole, or nearly the whole, re-appearing in the urine. At the same time, however, the nitrogen elimination usually falls below the level of the fasting state, sug-gesting that the sugar either undergoes partial oxidation and so spares the proteids, or its presence interferes with diffusion from the tissue cells, and so diminishes proteid destruction by raising the proportion of sugar in the blood. The effects of small quantities of sugar cannot be accurately estimated, owing to the natural variations in sugar excretion, and the influence of large amounts on the sugar excretion is complicated by the diarrhœa and intes-tinal disturbances that they produce.

Experiments have also been made with other forms of carbo-hydrate. The most interesting results are those that have been obtained with levulose, and substances containing it. When a large amount of levulose is given to a depancreatised dog, with a constant D : N ratio, a rise in the dextrose excretion occurs, and a small quantity of levulose appears in the urine ; but if only small quantities of levulose are given the increase in dextrose excretion is compara-tively slight, and no levulose is found. Examination of the glyco-gen content of the liver and muscles of such a dog shows a con-siderable amount to be present (8·14 per cent. in the liver, and 0·81 per cent. in the muscle in one case), so that, although the power to form glycogen from the dextrose has been lost, the power to convert levulose into glycogen is retained to a considerable extent. It is apparently only when more levulose is given than can be quickly converted into glycogen that the excess is excreted in the urine, partly as unchanged levulose, and partly as dextrose. That the glycogen in the liver and muscles comes from the levulose itself, is shown by the rise in the D : N ratio that follows its administration, readings of 11 to 13·5 being met with in some instances. With inulin,

which only yields levulose on hydrolysis, similar results have been
obtained. The administration of cane-sugar causes an increase
in the dextrose excretion corresponding to a little more than half
the sugar administered, showing that the whole of the dextrose,
but only part of the contained levulose, is excreted in the urine.
Since maltose on hydrolysis only yields dextrose, the sugar ex-
cretion is increased as though dextrose alone had been given.
Lactose very readily undergoes fermentation in the intestine, so that
exact observations are not possible, but it causes a considerable
increase in the dextrose excretion, and it is probable that this is
not only due to the contained dextrose, but that a certain amount
is derived from the galactose as well. Owing to the absence of
the secretions of pancreas from the intestine, starches are very
imperfectly digested, and appear to a large extent unchanged
in the fæces. A certain proportion is also broken down by the in-
testinal bacteria. The small part that is digested appears to be
absorbed in the usual way as dextrose, and consequently increases
the output of this sugar in the urine.

The effects produced by removing the pancreas have been
most thoroughly and completely investigated in dogs, but ana-
logous results have also been obtained with other members of the
vertebrate series, including cats and pigs (Minkowski and Harley),
carnivorous birds (Weintraud, Kausel, and Langendorff), frogs and
turtles (Aldehoff and Markuse), and eels (Capparelli). The pro-
portion of sugar in the blood has been shown by Kausel to be in-
creased in herbivorous birds by removing the pancreas, but glycos-
uria was found to only occasionally occur, probably because the
kidneys of these animals are not readily pervious to sugar. Ex-
periments on rabbits have usually been unsuccessful, owing to
the technical difficulties encountered in total extirpation of the
gland, which is spread out in a diffuse manner between the layers
of the mesentery ; but Hédon, and later Sauerbeck, succeeded in
producing atrophy, and transient glycosuria, by injecting oil into
the duct of Wirsung. The glycosuria appeared at the earliest
on the twentieth day, and was at its height from the thirtieth to
the thirty-eighth day after the injection.

Partial extirpation of the pancreas may, or may not, give rise
to glycosuria, according to the amount left behind, and its con-
dition. If about a fourth, or fifth, of the gland is left, glycosuria
only occurs if carbohydrates are present in the food (" alimentary
glycosuria "). A larger proportion usually prevents the condi-
tion. Less gives rise to frank diabetes. Even when sugar does
not appear in the urine after partial extirpation, it will do so if the

remnant is subsequently removed, and may gradually develop
as the fragment atrophies. Sandmeyer found that the first trace
of sugar appeared in the urine of a dog, part of whose pancreas
had been removed, seven weeks after the operation, but it was
not until after the lapse of thirteen and a half months that the
diabetes became permanent. Death occurred eight months later.
At the post-mortem a remnant of pancreatic tissue, weighing
0·36 gram, and showing no trace of glandular structure, was found
adherent to the posterior wall of the stomach, while attached to
the lower part of the duodenum was a piece of slightly changed
gland-tissue about the size of a pea. It has been remarked that
partial removal of the pancreas usually gives rise to more serious
polydipsia, polyphagia, and polyuria than total extirpation of the
gland.

The first explanation of the results of these experiments that
suggests itself is that removal of the pancreas leads to impaired
digestion from absence of the pancreatic juice from the intestine,
and that this is in some way responsible for the glycosuria. But
the fact that diabetic symptoms do not supervene unless almost
the entire gland has been removed is against such a theory. More-
over, if the secretion of the gland is diverted, and intestinal diges-
tion is thus prevented, diabetes does not follow, although marked
wasting may occur. Ligature of the pancreatic duct likewise fails
to give rise to glycosuria as a rule.

Disease of the solar plexus has been regarded by some as the
cause of the diabetes, and, as the solar plexus is almost unavoidably
injured in the removal of the pancreas, this might possibly be
the explanation of the symptoms caused by the depancreatisation
of animals. But it was shown by Minkowski that, if the descending
portion of the gland in a dog is transplanted into the subcutaneous
tissue of the abdominal wall and allowed to become engrafted
there, the intra-abdominal portion can be removed, after the graft
has been severed from its nervous connections, without producing
glycosuria, and that diabetes only develops if the graft is sub-
sequently removed, or atrophies. It is not improbable, however,
that the transitory glycosuria that follows immediately after partial
extirpation of the gland, and is sometimes seen as a consequence of
manipulation, or irritation, of the pancreas, or peri-pancreatic region,
may be of reflex nervous origin (Minkowski).

The cause of the hyperglycæmia, and the consequent glycosuria,
appears, therefore, to be the loss of some influence which the
pancreas exerts by way of the blood, or lymph, stream. It is
conceivable that this influence may be exerted in four different

ways : (1) The pancreas may destroy sugar coming to it in the blood ; (2) it may secrete an enzyme that destroys sugar in the blood ; (3) the cells of the pancreas may normally destroy, or modify, some toxic substance produced in other parts of the body which interferes with the utilisation of sugar by the tissues ; (4) the pancreas may produce an internal secretion which is necessary for the splitting up and use of sugars by the other cells of the organism.

1. As to the first possibility, that the pancreas normally destroys sugar coming to it in the blood, there is no evidence to support it. Numerous experiments have failed to show that the pancreas possesses greater glycolytic powers than other organs, and since only a small fraction of the gland, which may be merely engrafted in the subcutaneous tissue, is sufficient to prevent glycosuria, it is hardly conceivable that it is necessary for the bulk of the blood to actually transfuse the pancreas to be subjected to some glycolytic action.

2. The second hypothesis, that the chief function of the pancreas in carbohydrate metabolism is to furnish a glycolytic enzyme to the blood, is particularly associated with the name of Lépine, who has stoutly maintained the presence of such an enzyme in healthy blood, and states that it is diminished in depancreatised dogs. Crofton, who has supported Lépine's views, claims to have isolated the glycolytic enzyme, and to have identified it with trypsin. Other observers have found that, when precautions are taken against contamination, normal blood possesses no glycolytic power, and consider that contrary results have been due to post-mortem changes, and to the action of micro-organisms. Blumenthal and others have asserted that the cells of the pancreas, liver, spleen, muscle, &c., have strong glycolytic powers, which are much increased if pancreatic extract is mixed with the cell-juice of other organs ; but, according to Umber, when careful precautions are taken against contamination, the tissues outside the body exhibit only very slight glycolytic powers. The same observer also showed that the sugar-splitting power of the blood in the pancreatic vein is not greater than in the general arterial or venous system, as it should be if the pancreas secretes a sugar-destroying ferment.

3. The anti-intoxication theory is that which was at first favoured by Minkowski, but it was later abandoned by him in favour of the fourth hypothesis. He, and v. Mering, showed that if the blood of a depancreatised dog is transfused into a healthy animal glycosuria does not ensue, as it might be expected to do if the sugar excretion were dependent upon an accumulation of

toxic substances. Lombroso has proved that the glycosuria is not the result of an absorption of toxic substances from the intestine, formed in consequence of the defective pancreatic digestion, as had been suggested, by injecting fluid from a pancreatic fistula in one dog into the duodenum of another, depancreatised, animal. He found that, although the digestion of the second animal was much improved, the glycosuria was in no way affected. Tuckett, Bosanquet, and others have from time to time revived the anti-intoxication theory in a modified form. Tuckett has suggested that the pancreas normally forms an internal secretion which enters the circulation by way of the thoracic duct, and there neutralises a toxine absorbed by the lymphatics from the intestine during digestion. In support of this he states that if the thoracic lymph from a fasting dog is injected into the portal circulation of a cat, no hyperglycæmia, or glycosuria, results ; but that if the lymph from a dog during digestion is similarly injected, a hyperglycæmia, varying from 0·3 to 0·9 per cent., and a glycosuria, varying from 1·0 to 9·0 per cent., are produced. Confirmation of his results are, however, lacking. Bosanquet, in his Goulstonian lectures, favoured the view that diabetes is due to an increased internal dissociation of tissue (possibly fat) into sugar, caused by a toxic substance that is produced in the course of normal metabolism, and which is neutralised by the pancreas.

4. The remaining hypothesis, that the pancreas produces an internal secretion which, passing into the blood, brings about the destruction of dextrose elsewhere in the organism, is the one now most generally held, for it most readily and completely explains the phenomena resulting from the removal of the pancreas in animals.

Numerous attempts have been made to prove the existence of this internal secretion by administering preparations of the pancreas, or the fresh gland, by the mouth, by the injection of various extracts, and even of serum from the pancreatic vein, subcutaneously, or into the circulation, but they have all proved to be without effect on the glycosuria, so that it seemed that either the internal secretion of the pancreas did not exist, or that, as Pflüger suggested, the living organism supplies the circulating blood with its secretion so rapidly that there is never more than a trace in the gland at one time.

Evidence in favour of the latter suggestion was furnished by the experiments of Forsbach. This observer showed that if two dogs are united by skin, muscle, and peritoneum, so that an exchange of blood and lymph can take place through their com-

municating peritoneal cavities, and the pancreas of one is extirpated, the glycosuria that should occur is checked, but, on separating the depancreatised dog from the normal animal, sugar appears in the urine of the former to the same extent as usual. Hédon also found that when cross circulation is established between a normal and a diabetic dog, the sugar in the urine of the latter diminishes. When the experiment was repeated with two diabetic dogs, however, it was found that there was also a diminution in the glycosuria, a result which he interprets as being due to an alteration in the permeability of the kidneys for sugar.

Hédon subsequently carried out a further series of experiments, inserting a portion of the pancreas of a healthy dog into the circulation of a diabetic animal. He found that no effect was produced on the glycosuria when the carotid and jugular were used, but that when the pancreas was inserted into the circulation of the spleen the sugar almost disappeared after some hours, to return again when the connection between the two animals was severed, thus suggesting that the normal pancreas only checks glycosuria when it is so placed that its internal secretion enters the portal circulation directly. He also found that if blood from the pancreatic vein of a normal dog were injected into a mesenteric vein of a diabetic animal, the excretion of sugar fell, for a time, almost to the normal. Hédon considers that the first effect of the pancreatic secretion was to render the kidneys less permeable to sugar, for in all his experiments the sugar in the urine diminished more markedly than the sugar in the blood. These observations, although they need to be confirmed, tend to support the view that the pancreas influences carbohydrate metabolism by the production of an internal secretion, and also suggest that its action is to inhibit the production of sugar by the liver, rather than to facilitate its consumption by the tissues.

On the other hand, the recently published experiments of Knowlton and Starling, while they confirm the hypothesis that the pancreas controls sugar metabolism through an internal secretion, suggest that its presence is necessary for the utilisation of sugar by the tissues. Working with isolated heart-lung preparations, these observers found that the normal heart, fed with normal blood, under approximately physiological conditions, consumes about 4 mg. of sugar per hour per gram of heart muscle, but that the sugar consumption by the hearts of depancreatised dogs is practically nil, or at the least very much less. On feeding the heart from a diabetic dog with blood from a normal animal, it was found that, even in the first hour, the consumption of sugar was con-

siderably above that of a diabetic heart fed with diabetic blood, and steadily increased during the next two hours. In the reverse experiment, where a normal heart was fed with diabetic blood, the consumption of sugar during the first hour was only slightly below normal, but steadily diminished during the succeeding two hours. On adding a boiled extract of pancreas to the blood circulating through the heart of a diabetic animal, the sugar consumption was raised to a point approximating to that obtained with normal hearts. These experiments, therefore, suggest that the tissues and blood normally contain a substance derived from the pancreas, which is essential for the direct utilisation of sugar by the tissues. This substance, it would seem, is gradually used up in the process, and has to be continually replaced from the blood if the utilisation of sugar is to continue.

Cohnheim has stated that expressed muscle juice is inactive to sugar until it has been mixed with expressed tissue juice from the pancreas, which itself has only slight glycolytic properties. The products of the glycolysis were found to be carbon dioxide and water, if an abundance of oxygen was present ; but in the absence of oxygen, alcohol, then lactic acid, and finally oxybutyric acid are formed. He maintains that enough sugar destruction takes place in such experiments to fully account for the amount that is daily destroyed in the body. Sehrt confirms these results, and states that the combined pancreas-muscle, or pancreas-liver, extract does not destroy levulose as it does dextrose, thus accounting for the known ability of depancreatised animals to utilise levulose. Cohnheim explains the results he obtained on the lines of Ehrlich's side-chain theory. He argues that the muscles produce a ferment, which is itself incapable of decomposing sugar, but that, when acted on by an " activator substance " derived from the pancreas, it gains this power, in much the same way as complement is activated by amboceptor in hæmolysis and similar processes. The presence of blood in the muscle was found to bring about glycolysis without the addition of pancreatic extract, suggesting that the activator substance from the pancreas is present in the blood. Cohnheim states that a very small quantity of the activator substance is required, and that an excess interferes with its effects (cf. Ehrlich's " deviation of complement "). It is soluble in water and alcohol, but is insoluble in ether, so that an ether precipitate of the pancreatic extract may be used in the experiments. Since it is not destroyed by boiling he concludes that it is not a ferment, but is analogous in composition to adrenalin, iodothyrin, secretin, and other known products of internal secretion. Cohnheim's

conclusions have been supported by Arnheim and Rosenbaum, Hirsch, De Witt, and Hall, but have been criticised by Claus and Embden, and by Simpson, who doubt their value, and attribute some at least of the observed effects to contamination.

Even if Cohnheim's hypothesis were proved to be correct, and the decreased glycolysis met with as the result of pancreatectomy could be thus explained, it would not account for all the pathological changes that follow removal of the pancreas in animals, and particularly the failure of the liver and muscles to store glycogen and the increased amount of glycogen met with in abnormal situations. Von Noorden considers that the pancreas furnishes a ferment which favours the polymerisation of sugar into glycogen, or else an anti-ferment which prevents the too rapid destruction of glycogen. On account of the power which depancreatised dogs possess of forming glycogen from levulose, he inclines to the view that it is rather faulty formation of glycogen from grape-sugar, than its too rapid destruction that explains the defective glycogenesis. Wells suggests that either the glycolytic ferments or the glycogen are normally so combined in the liver cells that they cannot freely act, or be acted on, to form sugar, but that in the absence of the internal secretion of the pancreas this combination ceases.

Pavy advanced the view that the pancreas supplies a co-ferment or amboceptor which, by effecting the attachment of sugar to the bioplasmic molecule, places it in a position to be disposed of in accordance with the requirements of the existing environment. Normally it undergoes oxidation, and disappears with the liberation of energy, or it may be transmuted into glycogen, or transformed into fat. If, however, from absence, or disease, of the pancreas, the co-ferment is not produced the sugar molecule passes into the circulation in a free state, and glycosuria, proportional to the carbohydrate intake, results. The carbohydrate which has already been put into combination may also, under the influence of the abnormal environment, be split off, a disruption, similar to that which occurs in phloridzin glycosuria, taking place, so that the built-up molecule undergoes a series of changes the reverse of those that are naturally met with, and as a result a further amount of sugar appears in the urine.

An attempt to reconcile the discoveries of pancreatic glycosuria with the hepato-neurogenic theory of diabetes of C. Bernard was made by Chauveaux and Kaufmann. The source of the sugar according to their view is the liver. Normally the production of sugar by the liver is under the regulating influence of the nervous system and the pancreas, the nerves passing to the liver carrying

stimulating influences, while the pancreas has an inhibitory action on the formation of sugar. Originally they held the view that the influence of the pancreas was also exerted through the nervous system, but later altered this and suggested that an internal secretion was formed by the pancreas, and carried by the blood to the liver. When the pancreas is extirpated its inhibitory action in either case ceases, with the result that there is an over-production of sugar by the liver, and a consequent hyperglycæmia and glycosuria.

Boruttau has suggested that the adrenals produce a hormone which sets in motion glycogeny in the liver, while the pancreas furnishes another hormone in its internal secretion which antagonises the sugar-mobilising power of the adrenals.

Practically all observers of weight are now of opinion that the pancreas influences carbohydrate metabolism by the formation of an internal secretion, and all the available evidence points strongly in that direction, but, as we have just seen, there is considerable difference of opinion as to how it exerts its influence.

Another question which has not yet been settled, and on which there has been much controversy, is the exact seat of origin of the internal secretion, some hold that it is formed by the islands, or areas, of Langerhans, while others maintain that it is a product of the whole gland.

The suggestion that the islands of Langerhans are concerned in the production of the internal secretion of the pancreas was originally made by Laguesse in 1893. This view was subsequently adopted by Schäfer, Diamare, and others. The theory that such a relationship exists is based partly upon histological grounds, and partly on the results of experimental work, but the most important evidence in support of it has been furnished by pathological observations which suggest that pancreatic diabetes in man is due to a disturbance of the functions of the cell-islets.

Langerhans, in his description of the pancreas in 1869, was the first to draw attention to these characteristic structures now known as "intertubular cell-clumps," "interacinar islands," or "islands, or areas, of Langerhans." They consist of collections of small spherical or polygonal cells, which in man are apparently scattered irregularly through the gland substance, but in some animals occupy a definite position in the centre of the lobules. It is generally held that in adult life no connection exists between the cell islands and the duct system of the gland, but they are intimately related to the blood-vessels. The structure and relations of the cell islands has been the subject of numerous researches on

the part of a large number of investigators, who, while agreeing
on some points, differ in their description in many important
particulars. All those who have devoted attention to the subject
agree that very similar structures are found in all vertebrates, but,
while some regard them as permanent bodies endowed with special
functions, others look upon them as being of a temporary character,
and consider that they are in reality resting acini. The cells
composing the islands of Langerhans are always smaller than the
gland cells, each possesses a centrally placed round, or oval, vesicular
nucleus, which differs from those of the secreting cells in being
larger relative to the amount of cell protoplasm. The protoplasm
itself is very finely granular, does not stain at all with basic nuclear
dyes, and not well with acid stains, such as eosin. Lane has de-
scribed two types of cell, containing granules of different char-
acters, both of which differ in chemical nature from the zymogen
granules of the acinar cells. The islands are highly vascular, the
veins and capillaries forming a rich plexus of thin-walled vessels
(sinusoids) in intimate relation with the epithelial cells. The out-
line of the cell-islands is irregular, as De Witt has shown in her
reconstruction models. The digestion experiments of Flint have
demonstrated a well-defined capsule, connected on the one hand
with the alevolar framework, and on the other with septa which
stretch across the space within the island, dividing it into lacunæ,
and acting as a support for the cells of which it is composed. Ac-
cording to Flint, the connective tissue forming the framework has
a characteristic arrangement, in sharp contrast to that of the
remainder of the lobule. The size and distribution of the islands
is not uniform. Laguesse has distinguished five different types
varying from less than $100\,\mu$ in diameter to a giant form of over
$400\,\mu$ in diameter. Opie found that the cell-islets were more
numerous in the tail, or splenic end, than elsewhere in the human
pancreas, and he agrees with Kasahara that the pancreatic tissue
in the fœtus, and very young children, shows a larger number of
islands than that in the adult. This might be explained by the
islets being formed during embryological life and persisting un-
changed, while the secreting tissue increases in bulk.

The distribution of the islands in certain bony fishes is of con-
siderable interest and importance as bearing on the question of
their origin and significance. Rennie states that very large islands
are found in the areas of pancreatic tissue along the abdominal
vessels in all of the twenty-five species he investigated, and the
pancreas itself possesses no islets. In the pancreas of the guinea-
pig De Witt has described large, relatively isolated, cell-islets lying

in the connective tissue around the large ducts, especially about the junction of the splenic and middle thirds of the gland, and states that cell-islets, which appear to be free from pancreatic tissue, are also met with in the mesenteric fat near the periphery of the gland. The splenic portion of the pancreas of the cat, according to Opie, has constantly a cell-islet near the centre of each lobule. By means of what are claimed to be specific staining reactions, Bensley appears to have proved that the islands of Langerhans are special structures and not merely changed acinous tissue. He states that the total number of islands in the entire pancreas of the guinea-pig may vary between 15,000 and 45,000, the average number per milligram of pancreas working out in different animals at 9·5 to 189, and that an equally great variation in the number of islets exists between corresponding areas in different glands.

Light on the vexed question of the significance of these remarkable structures has been sought by a study of their development, but here again there is a difference of opinion. Hansemann believes that they arise from the interstitial tissue, and have no connection with the pancreatic acini. Laguesse described a double origin, the " primary islands," arising from the primitive pancreatic tubules, being permanent, and " secondary islands," developing from the acini, being transitory structures. Küster states that the cell-islets are derived from the ducts, and Helly believes that they are developed from the pancreatic tubules, early in embryological life, as solid outgrowths which subsequently become vascularised, and later develop a reticulum.

Experimental investigations have been conducted by a number of observers with a view to deciding the question of the individuality of the islands of Langerhans. Lewaschew maintained that stimulation of the pancreas by overfeeding, or by the administration of pilocarpine, causes the transformation of secreting acini into cell-islets, but Statkewitsch states that the changes observed are merely the results of intense activation in the gland cells, and are not stages in a transition to cell-islets. Jarotsky also came to the conclusion that the altered acini met with as the result of changed dietetic conditions are not connected with the islands of Langerhans. He attributes the results obtained by Lewaschew to imperfect fixation. Opie could detect no increase in the cell-islets after the administration of pilocarpine, and points out that in Lewaschew's experiments the normal variation in the number of cell-islets in different parts of the gland was not taken into account. De Witt, after studying the effects of starvation and various diets on the pancreas, came to the conclusion that, while some qualitative

changes are produced in the cell-islets, there are none that can be regarded as constant, or show a progressive increase with the duration of the experiment. Dale, and subsequently Laguesse, claim to have demonstrated that starvation increases the number of cell-islets, and Dale also states that the injection of secretin exhausts the acini, thereby converting many of them into islands of Langerhans. Vincent and Thompson, who have repeated Dale's experiments, confirm his conclusions. Schulze, Sauerbeck, Ssobolew, Zuntz and Mayer, De Witt, MacCallum, and Lombroso found that after ligature of the pancreas, or its ducts, the glandular acini degenerate, but that the islands of Langerhans are left intact. The experiments carried out by Schulze were repeated by Mankowski, who states that the pancreatic tissue disappears from between, and behind, two ligatures placed round the splenic end of the pancreas, while the cirrhosed portion in front shows as many, if not more, cell-islets than secreting acini, so that although the last-named result might suggest that the cell-islets are independent and more resistent structures than the acini, the same cannot be said of the remaining parts. It has been suggested by Hess that failure to permanently occlude all the ducts is the explanation of the persistence of groups of cells resembling cell-islets described by previous experimenters. Bearing this in mind, Pratt, Lamson, and Marks carried out a series of experiments with cats and dogs, and state that degeneration and destruction of both the acini and cell-islets, in constant and striking contrast to the results obtained by other investigators, occurs when the pancreatic secretion is completely excluded from the intestine by ligature of the ducts.

With so many conflicting results, and opinions, it is obvious that the question of the independence of the islands of Langerhans, and still more of their performing a special function, is as yet in an undecided condition, but from a review of the recent literature the impression is obtained that the case in favour of the cell-islets being separate tissues, with an independent function, is steadily growing stronger. In favour of this hypothesis are the constant presence of these structures at all ages, and in so many different animals, their early appearance in embryonic life, their different staining reactions, their peculiar arrangement, and their apparent independence of the duct system of the gland ; while their rich blood supply may be taken to indicate that they are possibly vascular glands engaged in the elaboration of an internal secretion, which is poured into the blood stream. It was their close resemblance to the para-thyroids and other ductless glands

that suggested to Laguesse that they were bodies concerned solely with the elaboration of an internal secretion. Those who believe that the islands of Langerhans are temporarily changed secreting acini, hold that the islets are closely related to the acini, from which, they contend, they are not separated by any definite capsule ; both structures have a common blood supply ; the islets can be shown to open into the pancreatic ducts ; in some sections various transition stages between typical acini and typical islets can be seen ; the number of islets is increased during activity of the gland, and diminished during rest ; by prolonged stimulation of the gland, either by overfeeding, by the administration of pilocarpine, or by the injection of secretin, it is possible to transform secreting acini into islands of Langerhans. Observers who favour this view maintain that the internal secretion is furnished by the acini, and that pancreatic diabetes is due to disease of those structures.

The most strenuous opponent of the theory that the glycosuria produced by extirpation of the pancreas is dependent upon the lack of an internal secretion formed by the gland has been Pflüger, who asserts that the pancreas is under the control of the nervous system, and that in the wall of the duodenum there exists an " anti-diabetic " centre, rich in ganglion cells, which, by nerves passing to the gland, controls its anti-diabetic powers, so that the pancreatic diabetes of von Mering and Minkowski is in reality a " duodenal " diabetes, due to destruction of these nerves. Experimenting with frogs, Pflüger found that extirpation of that portion of the duodenum which is in contact with the pancreas was followed by severe diabetes, and that even cutting the nerves which pass from the duodenum to the pancreas produced the same effect. His experiments with dogs were not quite so satisfactory, owing, he states, to the difficulty of the operation and the speedy death of the animal ; but even with these Pflüger found that excision of the duodenum produced glycosuria. His conclusions with regard to dogs and other mammals have been disproved by Ehrman, Lauwens, Rosenberg, Minkowski, and Pratt, who showed that total removal of the duodenum, or section of the nerves passing from the duodenum to the pancreas, does not give rise to glycosuria. Rosenberg and Lowitt have also demonstrated that " duodenal " diabetes does not exist in the frog, and that the glycosuria observed by Pflüger was probably produced by keeping his animals on ice.

According to Ott, the injection of fresh pancreas into the jugular vein of animals produces slight glycosuria, about a quarter per

cent. of sugar usually appearing in the urine. Leschke states that pancreatic extracts derived from frogs or guinea-pigs, whether fresh, inactivated by heating to 70° C. for a quarter of an hour, or after being heated to 100° C. for ten minutes, when injected subcutaneously, or intravenously, into normal guinea-pigs or frogs, give rise to glycosuria. When injected into animals with pancreatic diabetes they increase the glycosuria, at the same time occasioning a severe disturbance of the general condition, which shortly ends in death.

In spite of the conflicting results of many of the observations on the relation of the pancreas to glycosuria, and the existence of different theories as to the way in which the gland controls the internal carbohydrate metabolism of the body, certain conclusions stand out as being generally accepted. These may be summarised as follows :—

(1) Total extirpation of the pancreas always gives rise to fatal diabetes.

(2) The presence of at least a fourth or fifth of the gland in a healthy condition prevents glycosuria.

(3) Transplantation of a portion of the pancreas prevents the development of diabetes when the rest of the gland is removed.

(4) The conclusion is justified that the pancreas is concerned with the internal metabolism of sugar.

Exactly how it exerts its metabolic functions, the relation of the islands of Langerhans to the acini, and the relative importance of the islands and the acini in carbohydrate metabolism, are questions that are as yet unsettled, and await more conclusive evidence for their solution.

(*b*) **The Supra-renals.**—In 1901 Blum announced that the subcutaneous injection of an aqueous extract of the supra-renal capsules produces transitory glycosuria in dogs. Subsequently Bierry and Gatin-Gruzewska, Patta, Lépine, and others confirmed his results, and showed that intravenous, or intra-peritoneal, injections of extracts of the glands, or of adrenalin chloride, give a more marked result, and also more quickly. Massage of the exposed supra-renal capsules was found by Herter to produce glycosuria, while most minute quantities of adrenalin applied directly to the surface of the pancreas cause the appearance of sugar in the urine. The dose of epinephrin given in all experimental work must, however, be enormous compared with the amount normally poured into the blood, and Ritzmann states that it is only when

the quantity of epinephrin administered is sufficient to produce a rise in blood pressure that glycosuria results. When a sufficiently dilute solution (1,000,000 to 2,000,000) is used, it can be allowed to flow into a vein at the rate of two cubic centimetres a minute without sugar appearing in the urine ; but if the solution is more concentrated, or the dilute solution is run in faster, glycosuria follows.

The injection of adrenalin greatly intensifies the glycosuria in depancreatised dogs, and it has been shown by Fronin and Mayer that, on the other hand, the extirpation of the adrenals prevents the glycosuria that would otherwise result from removal of the pancreas.   According to Mayer, extirpation of the adrenals also prevents the appearance of sugar after diabetic puncture.   Porges has shown that, after the extirpation of both supra-renals in dogs, only a small amount of glycogen is present in the liver and muscles, and that the quantity of sugar in the blood is lowered.   Falta states that high section of the spinal cord, or section of the nervous connections of the adrenals with the diabetic centre, leads to a very marked reduction in the amount of sugar in the blood.   Pollak found that the administration of adrenalin causes glycosuria when both splanchnics have been cut.

The blood of animals with glycosuria produced by adrenalin injections has been examined by Zuelzer and Metger, who found that it contains an excess of sugar, so that the immediate cause of the appearance of sugar in the urine, as in pancreatic and puncture diabetes, is a hyperglycæmia.   Since the most striking physiological action of adrenalin is its vaso-constricting power, it would seem not unlikely that the hyperglycæmia and glycosuria might result from its effect on the circulation, the pancreas, &c., being rendered anæmic, and thus functionally inactive.   Marked vaso-constriction may, however, be induced in guinea-pigs by the administration of supra-renal extract, without glycosuria resulting, and, as we have seen, the injection of adrenalin intensifies the glycosuria of depancreatised animals.   The very marked effect of the direct application of adrenalin to the pancreas has also suggested that it may act directly upon the gland and interfere with its control of sugar metabolism ; but here again the results of injections into animals deprived of their pancreas seemed to negative such an idea, at least in its entirety.   It was shown by Iwanoff that adrenalin increases the rate of discharge of sugar from the glycogen-rich liver, through which salt solution is being transfused, suggesting that the internal secretion of the supra-renals acts directly upon the glycogen splitting enzyme of the liver cells ;.

but starved rabbits, from whose tissues most of the glycogen has been removed by repeated doses of strychnine, have been shown by Pollak to react to adrenalin, so that an increased production of dextrose from glycogen cannot be the sole explanation of the hyperglycæmia and glycosuria. Underhill found that piperidine produces similar results to adrenalin, but the glycosuria induced by piperidine and certain other chemicals, such as potassium cyanide, ether, chloroform, morphine, strychnine, curare, &c., differs from that produced by adrenalin, inasmuch as the former is prevented by the administration of oxygen, while the latter is not. Elliott has pointed out that a characteristic of epinephrin is its tendency to stimulate plain muscle and gland cells that are, or have been, in functional union with sympathetic nerve fibres. In harmony with this fact Underhill and Clossen have suggested that the internal secretion of the supra-renals acts upon the liver and other storehouses of carbohydrates through the sympathetic nervous system, causing them to throw out the carbohydrate they contain in the form of dextrose, while at the same time there is diminished storage from stimulation of the hepatic cells, which reject the dextrose brought to them by the blood. From the similarity of the effects produced by adrenalin injections and diabetic puncture, and the increase in the blood of adrenalin that the latter is said to produce, Schur and Wiesel have inferred that the sympathetic fibres actuate the formation of sugar in the liver through the adrenals.

Zuelzer, Dohran, and Marxer have described extensive experimental researches which seem to show that adrenalin plays an active part in the mobilisation of sugar both in physiological conditions and in pancreatic diabetes. They conclude that adrenalin, and the hypothetical internal secretion of the pancreas, have an antagonistic action, and that it is by their combined effect on the liver that sugar metabolism is regulated.   In pancreatic diabetes the lack of the pancreatic secretion, and the consequent predominance of adrenalin, explains the increased output of sugar.   When both the pancreas and the supra-renals are incapable of functioning, diabetes does not occur.   Zuelzer states that he has extirpated the pancreas from more than a hundred animals, and that pronounced glycosuria invariably followed, excepting in those cases where adrenalin was excluded from the circulation by ligaturing the veins from the supra-renals.   Underhill and Fine have found that hydrazine prevents the glycosuria occurring after extirpation of the pancreas, probably because this drug diminishes adrenal activity.

All these researches tend to show that the adrenals exert an

K

important influence on carbohydrate metabolism through their internal secretion. Physiologically their task appears to be to mobilise the sugar from the liver, and probably also from the other tissues, possibly through the intermediation of the sympathetic nervous system. That a body of known composition, such as adrenalin is, should perform such an important function is most interesting, and furnishes a noteworthy example of the specific action of an optically inactive substance containing an asymmetrical carbon atom. It will be remembered, however, that adrenalin is the secretion of the medullary, or chromaffin tissue, of the supra-renal capsules only, and that chromaffin tissue is also found in other situations, although in very much smaller amounts.

(c) **Pituitary Gland.**—It has long been known that glycosuria is one of the most frequent symptoms of acromegaly, and that in such cases overgrowth of the pituitary gland is found after death. In the group of symptoms known as " Fröhlich's syndrome " or " dystrophia adiposo-genitalis," which is believed to result from hypo-pituitarism, on the other hand, glycosuria is almost unknown, although thirst and polyuria are common.

Experiments on animals have pointed to the hypophysis as being concerned in the metabolism of sugar. After destroying the posterior, without touching the anterior, lobe in a dog, Caselli found that sugar appeared in the urine. Polyuria and sometimes glycosuria occur in animals after partial removal of the anterior, and total removal of the posterior, lobe according to Cushing. It has been shown by Borchardt that the hypodermic injection of a boiled, filtered infusion of the gland into rabbits and dogs causes a small amount of sugar to appear in the urine, varying from a trace to 4·2 per cent. The sugar usually began to appear in the urine about three hours after the injection, but the total amount excreted was very small, generally not more than a centigramme, and was extraordinarily variable. Glycosuria was found to be more readily produced in rabbits than in dogs, but in both the amount of sugar excreted appeared to be independent of the quantity of gland extract injected. Ott and Scott injected 1 c.c. of a 20 per cent. extract of the pituitary (" Infundibulin ") into the muscles of rabbits, and found, in all cases, at the end of two and a half hours about $\frac{1}{8}$ per cent. of sugar in the urine. Intramuscular, and intraperitoneal, injections into cats produced similar results. Section of the splanchnin nerves was found to arrest the glycosuria, which they suggest indicates that infundibulin acts on the diabetic centre in the medulla by way of the splanchnics.

The relation of the pituitary gland to carbohydrate metabolism has been very thoroughly investigated in a series of experiments carried out by Goetsch, Cushing, and Jacobson. These authors, like their predecessors, come to the conclusion that it is the posterior lobe (*pars nervosa et intermedia*) that is particularly concerned with this function. They state the secretion of the posterior lobe is discharged into the cavity of the third ventricle, and becomes dissolved in the cerebro-spinal fluid, a medium which passes from the ventricles to the subarachnoid spaces, and thence, in all probability, enters the blood-stream by way of the dural sinuses. Under various forms of operative manipulation of the infundibulum and hypophyseal stalk-structures, which appear to hold the reserve deposit of posterior lobe secretion, a transient hyperglycæmia is produced, presumably due to the setting free of an excess of this secretion, which in turn causes the discharge of stored glycogen. For the succeeding few days the assimilation limit for ingested carbohydrates is considerably diminished, alimentary glycosuria being produced by a smaller amount of sugar than was previously required. If the operation has been so conducted as to create a subsequent and permanent insufficiency of posterior lobe secretion (either owing to the removal of a considerable portion of this lobe with its epithelial investment, or thorough interference with its secretory discharge, either by placing a " clip " on the stalk, or by so damaging it that an infundibular cicatrix forms), the temporary lowering of the assimilation limit is succeeded by an abnormal, and enduring, augmentation in the tolerance for sugars. The assimilation limit for carbohydrates, greatly increased under these circumstances, can be promptly lowered by the coincident intravenous or subcutaneous injection of posterior lobe extract. This extract, furthermore, has a pronounced effect in lowering the sugar tolerance of the normal animal, in whom it may even cause glycosuria when given in sufficiently large doses.

(*d*) **The Thyroid.**—Experimental and clinical evidence both suggest that the thyroid gland is an essential factor in the regulation of the metabolism of the body, and that in some way it is connected with the utilisation of carbohydrates.

McCurdy, Falta, and others have found that extirpation of the thyroid raises the tolerance of animals for sugar, so that alimentary glycosuria is produced with difficulty, provided that the parathyroids are preserved. The administration of adrenalin is said by Falta not to produce glycosuria in thyroid-ectomised dogs,

although Underhill found that, if a sufficiently large dose is given, sugar may appear in the urine. MacCallum showed that the glycosuria following pancreatectomy is diminished by extirpation of the thyroid. After extirpating the thyroid in etherised cats, leaving two or more parathyroids, Ott and Scott found sugar in the urine on the following day in a few cases. On injecting infundibulin into the jugular vein the amount of sugar was diminished, as compared with animals whose thyroid was intact, the injection causing the appearance of 3 to 4 per cent. of sugar in the urine before removal of the thyroid, and 1 to 2 per cent. after. King states that the addition of thyroid juice to the Cohnheim pancreas-muscle-dextrose mixture markedly diminishes glycolysis.

A microscopical examination of the thyroids of three dogs in which chronic pancreatic insufficiency had existed for from five to thirty-four months, by Pratt, showed a partial or complete replacement of the colloid material by desquamating epithelial cells, so that the structure of the gland was scarcely recognisable, suggesting a compensatory activity of that organ.

The influence of the thyroid on carbohydrate metabolism has also been noted in clinical medicine. In exophthalmic goitre (hyperthyroidism) glycosuria is often present, and even if it is not, it is readily induced by feeding with large doses of sugar. The continued use of thyroid preparations has also occasionally been followed by diabetes. In myxœdema (hypothyroidism), on the other hand, glycosuria is practically never met with, and even large doses of sugar can be taken without any being excreted in the urine.

How the thyroid influences carbohydrate metabolism is not yet decided. Opie inclines to the view that the glycosuria of Grave's disease is due to an associated pancreatic lesion, and such lesions have been found in fatal cases (Cecil). The fact that thyroid administration may produce glycosuria can, however, be only reconciled with such an explanation by supposing that hyperthyroidism gives rise to changes in the pancreas. Falta has suggested that an excess of thyroid secretion exercises a check upon the functions of the pancreas, either directly, or by stimulating the activity of the chromaffin system—that is to say, of tissues such as the medulla of the supra-renals, &c., which contain cells that readily stain with chromic acid and its salts. In favour of the latter suggestion are the observations of Kraus and Friedenthal, and of Fränkel, who detected an increase of adrenalin in the blood of patients with Grave's disease. Falta has also extended the observations of Schmidt and Salomon on the fæces in exoph-

thalmic goitre, and points out that the occurrence of fatty stools suggests a coincident restriction of the external secretion of the pancreas. If hyperthyroidism tends to induce glycosuria by exercising a restraining influence upon the functions of the pancreas, rather than by causing lesions of the gland, we should expect to find that a withdrawal of the normal supply would have the opposite effect, and that the pancreas, relieved of its wonted restraint, would be unusually active as regards carbohydrate metabolism. That is to say, hypothyroidism would increase the tolerance for carbohydrates, and this, as we have seen, is the case. Moreover, it has been found that the administration of thyroid extract lowers the tolerance again. There is thus a very striking resemblance between the effects of alterations in the functions of the thyroid and the posterior lobe of the pituitary body, and it seems probable that the mechanism is in both cases the same, or similar, especially as it is known that removal of the thyroid leads to hypertrophy of the pituitary.

(e) **The Parathyroids.**—The parathyroids appear to have an opposite influence to the thyroid on carbohydrate metabolism (Eppinger, Falta, and Rudinger). Underhill and Hilditch found that, after the excision of three of the four parathyroids of a dog, the tolerance of the animal for sugar was greatly lowered, and that complete thyreo-parathyroidectomy, besides causing tetany, &c., gave rise to glycosuria. Ott states that after the injection of the nucleo-protein of the parathyroids into a vein, sugar appears in the urine, and that large doses of parathyroid subcutaneously produce glycosuria in rabbits.

Theory of the Correlation of the Ductless Glands.—It has been pointed out by Eppinger, Falta, and Rudinger, who have themselves made numerous experiments and added much to our knowledge of the functions of the ductless glands, that the removal of a gland with an internal secretion acts in two ways. There is, first, the direct result of the absence of its secretion, and, secondly, the indirect effect due to the disturbance of metabolism consequent on its relations to other glands. They consider that the ductless glands may be arranged in two groups, according to the disturbance in the metabolic functions of the body to which alterations in their secretory activities may give rise. Broadly, they may be divided into (1) an accelerator group and (2) an inhibitory group. To the former class belong the thyroid, the pituitary body, and the supra-renals, since a study of the reactions of the body to injections of preparations from these organs, and observations

on the results of experimental interference with their functions,
suggest that all three increase protein exchange, the adrenals
cause mobilisation of carbohydrates, and the thyroid causes in-
creased fat destruction. Into the second group fall the pancreas
and the parathyroids, since both retard protein destruction, the
pancreas being the more active, and both restrain the mobilisation
of carbohydrates ; here again the pancreas is more active. The
pancreas also causes a decrease in fat consumption. These ob-
servers hold that the internal secretions of the pancreas and thyroid
mutually inhibit each other's activities, and that there is a similar
mutual inhibitory action between the pancreas and the chromaffin
system of the body, while between the thyroid and the chromaffin
system there is reciprocal promotion of action.

This inter-relation may be schematically represented by a
quadrilateral figure, with the pancreas, thyroid, parathyroids, and
chromaffin tissue at the four angles :—
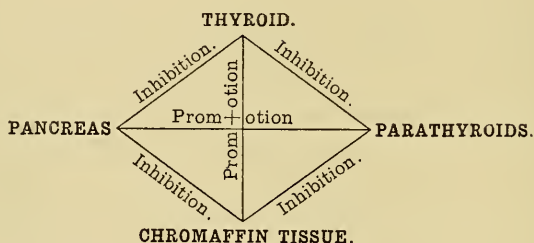
It is believed that all the ductless glands act through the
discharge of specific hormones into the circulation, and that in the
case of epinephrin it has a special affinity for the sympathetic
nerves. The mobilisation of sugar in the liver is assumed to be
under the influence of the sympathetics, by mediation of the supra-
renals, while the pancreatic internal secretion has an opposite effect
to epinephrin, inhibiting the mobilisation of sugar in the liver
and other tissues. The action of the two secretions is thus anta-
gonistic. The secretion of the thyroid is believed to inhibit the
activity of the pancreas and to promote that of the chromaffin
system in carbohydrate metabolism, the inhibition being, however,
stronger than the promotion.

According to this view the glycosuria seen after extirpation
of the pancreas may be looked upon as a negative pancreatic
diabetes and a positive adrenal diabetes, for the normal inhibitory
action of the internal secretion of the pancreas is removed, while

the mobilising power of the adrenal secretion is increased by hyperfunction of the chromaffin tissue. The latter results partly from the removal of the inhibitory action that the pancreas normally exerts on the adrenals, &c., and partly as an indirect effect of the absence of the similar inhibitory action that the pancreas has on the thyroid, which is thus at liberty to exert its stimulating action on the chromaffin tissue unchecked. At the same time there is excessive metabolism of proteids, fats, and carbohydrates as a consequence of the hyperfunction of the thyroid. This explanation would account for the greater intensity of pancreatic as compared with other forms of experimental diabetes.

The glycosuria following the injection of adrenalin can be regarded as a direct result of the rapid and excessive mobilisation of carbohydrates arising from hyperfunction of the chromaffin tissue, and indirectly as a result of an increased inhibition of the functions of the pancreas. Owing to the simultaneous promotion of the action of the thyroid that occurs, there is also an increased metabolism of proteids and fats. If the pancreas has been previously removed this is more marked, and the dextrose to nitrogen ratio in the urine is raised.

Removal of the thyroid leads to hyperfunction of the pancreas, owing to the removal of its inhibitory action on that gland, while at the same time it diminishes adrenal action, from the absence of the promoting effect of the thyroid on the chromaffin tissue. Hyperthyroidism produces the opposite effects, causing relative pancreatic insufficiency, and increased adrenal activity, with a tendency to glycosuria.

Extirpation of both the thyroid and pancreas removes at the same time the inhibitory effect of the pancreas and the promoting action of the thyroid on the chromaffin tissue, but as, according to Falta, the former is stronger than the latter, the mobilisation of fats is increased ; owing to the absence of the thyroid, however, their destruction is decreased. Under these circumstances sugar is formed from fat, and there is a raised dextrose to nitrogen ratio in the urine.

The glycosuria following puncture of the floor of the fourth ventricle is brought into line by the explanation, to which reference has been made, that the increased output of sugar by the liver on which it depends, arises from an excessive flow of epinephrin, brought about by impulses transmitted to the supra-renal capsules through the left sympathetic nerve.

It is possible that a number of toxic influences also cause the appearance of sugar in the urine by their action on the supra-

renals, either directly, or through the medium of the sympathetic nervous system or the diabetic centre in the medulla.

The views just outlined are to some extent theoretical, and this is particularly so as regards the part the chromaffin system plays, but they correlate, in a much more satisfactory manner than has previously been possible, the data that have accumulated on experimental glycosuria, with the exception of phloridzin glycosuria, which appears for many reasons to stand in a class apart. Whether subsequent observation confirms, or in part disproves, these theories there can be no doubt that they open up a much wider conception of the pathology of diabetes, and indicate that glycosuria is not a disease, but a symptom common to many pathological conditions.

BIBLIOGRAPHY

Achard, *Zeit. f. klin. Med.*, xvi.

Achard and Delamare, *Thése de Paris*, 1899.

Aldehoff, *Zeit. f. Biol.*, 1892.

Arnheim and Rosenbaum, *Zeit. f. phys. Chem.*, 1904.

Asher, *Zeit. f. Biol.*, 1912.

Bayliss and Starling, *Proc. Roy. Soc.*, cxix., lxxiii.

Bensley, *Amer. Journ. of Anat.*, 1911.

Bernard, *Compt. Rend. d. l. Acad. d. Sci.*, 1849.

Biel and Kolisch, quoted Pasteau *Jr. Med. Français*, 1911.

Bierry and Gatin-Gruzewski, *Compt. Rend. d. l. Soc. d. Biol.*, 1905.

Blum, *Deut. Arch. f. klin. Med.*, 1901.

Blumenthal, *Zeit. f. diat. u. physik. Therap.*, 1898.

Bock and Hoffman, *Reichert u. du Bois Raymond's Arch.*, 1871.

Borchardat, *Zeit. f. klin. Med.*, 1908.

Boruttau, quoted Ott, *Internal Secretions*, 1910.

Bosanquet, *Lancet*, 1905, 1908.

Bouchardat, *De la Glycos. et Diab. Sucré*, 1875.

Capparelli, *Arch. ital. d. Biol.*, 1894.

Carnot, *Malad. d. Gland. Saliv. et d. Pancreas*, 1908.

Caselli, quoted Ott, *Internal Secretions*, 1910.

Cecil, *Journ. Exp. Med.*, 1909.

Chauveaux and Kaufmann, *Compt. Rend. d. l. Soc. d. Biol.*, 1893–4.

Chittenden and Lambert, *Zeit. f. Biol.*, 1889.

Claus and Embden, *Beitr. z. chem. Physiol. u. Path.*, 1905.

Cohnheim, *Zeit. f. physiol. Chem.*, 1903–4–6.

Crofton, *Amer. Journ. Med. Sci.*, 1902.

Dale, *Proc. Roy. Soc.*, 1904.

Diamare, *Internat. Monat. f. Anat. u. Path.*, 1899.

Ehrmann, *Arch. f. d. ges. Physiol.*, 1906 ; *Zeit. f. exp. Path.*, 1908.

Elliott, *Journ. Physiol.*, 1905.

Eppinger, Falta, and Rudinger, *Zeit. klin. Med.*, 1908–9.

Falta, *Wein. klin. Woch.*, 1907 ; *Cong. f. inn. Med.*, 1907 ; *Zeit. f. klin. Med.*, 1910.

Fischer, *Pflüger's Arch.*, 1905.

Flint, *Arch. f. Anat. u. Entwickl.*, 1903.

Forsbach, *Arch. f. exp. Path. u. Pharm.*, 1909.

Fronin and Mayer, *Cong. f. inn. Med.*, 1908.

Goetsch, Cushing, and Jacobson, *Johns Hopkins Hosp. Bull.*, 1911.

Hall, *Amer. Journ. Physiol.*, xviii.

Hansemann, *Zeit. f. klin. Med.*, 1894 ; *Verhand. d. deut. path. Gesellsch.*, 1902.

Harley, *Journ. Anat. and Physiol.*, 1891–2 ; *Brit. Med. Journ.*, 1892.

Hédon, *Compt. Rend. d. l. Soc. d. Biol.*, 1911.

Helly, *Arch. f. mik. Anat.*, 1905.

Herter, *Med. News*, 1902.

Hess, *Münch. med. Woch.*, 1902 ; *Arch. f. d. ges. Physiol.*, 1907.

Hirsch, *Beitr. z. chem. Physiol. u. Path.*, 1904.

Iwanoff, *Centr. f. Physiol.*, 1905.

Jarolzky, *Virchow's Arch.*, 1899.

Kasahara, *Virchow's Arch.*, 1896.

King, quoted Ott *Internal Secretions*, 1910.

Knowlton and Starling, *Lancet*, 1912.

Küster, *Arch. f. mik. Anat.*, 1904.

Laguesse, *Compt. Rend. d. l. Soc. d. Biol.*, 1893, 1910 ; *Journ. l'Anat. et Physiol.*, 1894.

Lane, *Amer. Journ. Anat.*, 1907.

Langerhans, *Inaug. Dissert.*, Berlin, 1869.

Lauwens, *Arch. f. d. ges. Physiol.*, 1907.

Lépine, *Diabète Sucré*, 1909.

Leschke, *Arch. f. Anat. u. Physiol.*, 1910 ; *Chem. Zentralb.*, 1911.

Lewaschew, *Arch. f. mik. Anat.*, 1886.

Lombroso, ref. *Biochem. Centralb.*, 1903 ; *Journ. d. Physiol. et Path. gen.*, 1905.

Löwitt, *Arch. f. exp. Path. u. Pharm.*, 1910.

Lusk, *Science of Nutrition*, 1906.

MacCallum, *Johns Hopk. Hosp. Bull.*, 1909 ; *Journ. Amer. Med. Ass.*, 1910.

MacLeod, *Recent Advances in Physiol.*, 1906 ; *Journ. Amer. Med. Ass.*, 1910.

Mankowski, *Arch. f. mik. Anat.*, 1901.

Mayer, *Compt. Rend. d. l. Soc. d. Biol.*, 1906.

Mendel and Lusk, *Deut. Arch. f. klin. Med.*, 1904.

Von Mering, *Cong. f. inn. Med.* 1886 ; *Zeit. f. klin. Med.*, 1888–9.

Von Mering and Minkowski, *Centralb. f. klin. Med.*, 1889 ; *Arch. f. exp. Path.*, 1890 ; *Berl. klin. Woch.*, 1892.

Metzger, *Münch. med. Woch.*, 1902.

Minkowski, *Berl. klin. Woch.*, 1890, 1892 ; *Centralb. f. klin. Med.*, 1890 ; *Arch. f. exp. Path. u. Pharm.*, 1893, 1908.

Von Noorden, *Diabetes Mellitus*, 1906.

Opie, *Journ. exp. Med.* 1901 ; *Diseases of the Pancreas*, 1903.

Ott, *Internal Secretions*, 1910.

Ott and Scott, *Internal Secretions*, 1910.

Pal, *Wien. med. Presse*, 1898 ; *Wiener klin. Woch.*, 1902.

Patta, *Arch. ital. d. Biol.*, 1906.

Pavy, *Lancet*, 1908.

Pavy, Brodie, and Siau, *Lancet*, 1903.

Pearce, *Amer. Journ. Anat.*, 1903.

Pflüger, *Arch. f. d. ges. Physiol.*, 1907–8.

Pollak, *Arch. f. exp. Path. u. Pharm.*, 1909.

Porges, *Berl. klin. Woch.*, xxv.

Pratt, *Journ. Amer. Med. Assoc.*, 1910, 1912.

Pratt, Lamson, and Marks, *Tr. Assoc. Amer. Phys.*, 1909.

Reilley, Nolan, and Lusk, *Amer. Journ. Physiol.*, 1898.

Rennie, *Quart. Journ. Micros. Sci.* 1904 ; *Zeit. f. Physiol.*, 1905.

Richter, *Deut. med. Woch.*, 1899.

Ritzmann, *Arch. f. exp. Path. u. Pharm.*, 1909.

Rosenberg, *Arch. f. d. ges. Physiol.*, 1908 ; *Biochem. Zentralb.*, 1909.

Sandmeyer, *Zeit. f. Biol.*, 1892–4.

Sauerbeck, *Virchow's Arch.*, 1904 ; *Centralb. f. path. Anat.*, 1904.

Schäfer, *Lancet*, 1895 ; *Brit. Med. Journ.*, 1895.

Schulze, *Arch. f. mik. Anat.*, 1900.

Schur and Wiesel, quoted Ott *Internal Secretions*, 1910.

Sehrt, *Zeit. f. klin. Med.*, 1905.

Simpson, *Biochem. Journ.*, 1910.

Ssobolew, *Virchow's Arch.*, 1902.

Statkewitsch. *Arch. f. exp. Path. u. Pharm.*, 1902.

Stiles and Lusk, *Amer. Journ. Physiol.*, 1903.

Tuckett, *Journ. of Physiol.*, 1899.

Umber, *Zeit. f. klin. Med.*, 1900.

Underhill, *Journ. Biolog. Chem.*, 1905–6.

Underhill and Clossen, *Cent. f. Physiol.*, 1905.

Underhill and Fine, *Journ. Biolog. Chem.*, 1911.

Underhill and Hilditch, *Amer. Journ. of Physiol.*, 1897.

Vincent and Thompson, *Internat. Monat. f. Anat. u. Physiol.*, 1907.

Visentini, *Arch. f. Anat. u. Physiol.*, 1908.

Weintraud, *Arch. f. exp. Path.*, 1894.

Wells, *Chem. Pathology*, 1907.

De Witt, *Journ. of Exp. Med.*, 1906.

Zuelzer, *Berl. klin. Woch.*, 1901.

Zuelzer, Dohrn, and Marxer, *Deut. med. Woch.*, xxxiv.

Zuntz, *Arch. f. Physiol.*, 1895.

Zug and Mayer, *Neen. d. l. Acad. roy. d. med. Belgique*, 1906.

CHAPTER V

IT is well to remember that glycosuria is in reality but a relative term, and that the presence of sugar in the urine does not necessarily denote a pathological condition. The urine of a healthy individual, taking an average amount of exercise and food, contains at most a mere trace of sugar, that can only be detected by special methods ; but when a large quantity of sugar, and particularly sugar in solution (for example, sweet wine, &c.), is consumed, an amount of dextrose that is demonstrable by ordinary clinical tests may appear in the urine. This may be termed physiological glycosuria, for it is transitory, is not associated with any signs of disease, and is but an exaggeration of the normal condition.

The limit up to which a healthy person can completely utilise sugar varies with the individual, the conditions under which it is taken, and the nature of the sugar. Moritz found 0·2 to 0·3 per cent. of sugar in the urines of four out of six healthy people who had taken a large quantity of sweets and sweet champagne, but the urines of the other two were sugar-free. A smaller dose of sugar usually gives rise to glycosuria when taken on an empty stomach than when consumed after a full meal. The assimilation limit also varies with the time of day, being higher in the early morning than at night. Muscular work raises the power of assimilation, thus Bruel found that an individual who excreted 2·14 grams of sugar, in four hours after taking 200 grams, when at rest, only passed 0·09 grams after the same amount when doing muscular work. Hofmeister showed that the assimilation limit is lower for the starving organism than for the well-fed one. Young persons can as a rule dispose of a larger amount of sugar without sensibly affecting the urine than adults. Bouchard quotes the case of a boy of seventeen who consumed daily, for over five years, 600 grams of cane-sugar (about 13 grams per kilo of his body-weight) without producing glycosuria. The appearance of alimentary glycosuria seems to be favoured by alcoholic drinks. As a rule sugar begins to appear in the urine about half to one hour after it has been

taken, and reaches its maximum in about two to four hours. With a moderate dose the excretion ceases in about eight to ten hours.

To explain physiological alimentary glycosuria it is generally assumed that assimilation does not keep pace with absorption, owing to the power of the liver to form and store glycogen being limited. Hence, if too large an amount of sugar is taken into the portal system in a short space of time, only a part can be intercepted and removed by the liver, the remainder passing into the systemic circulation. As soon as this happens sugar appears in the urine, for the kidneys excrete any excess over the normal limit of about 0·1 per cent. that may be present in the blood. Ginsberg has suggested that when a large quantity of sugar is present in the intestine some may be absorbed by way of the lacteals and thoracic duct, thus escaping the influence of the liver, and so increasing the percentage of sugar in the blood.

Dextrose has the highest assimilation limit, 150 to 200 grams in a single dose ; for *levulose* it is a little lower, 140 to 160 grams ; *cane-sugar* has about the same assimilation limit as dextrose, 150 to 200 grams ; but for *lactose* it is lower, 120 grams with some individuals, and as little as 60 grams for others. The *pentoses* can only be assimilated to a very moderate degree, for when as little as 30 to 50 grams are given by the mouth, almost half reappears in the urine. *Starch* can be taken in very large quantities by a healthy individual without producing glycosuria, apparently be- cause its hydrolysis and absorption in the intestine take place so gradually that the assimilative powers of the liver, and other organs, are able to keep pace with the sugar as it enters the circulation. Rubner found that a man consuming a daily ration of 508 to 670 grams of carbohydrate, in the form of wheaten bread, left un- absorbed only 0·8 to 2·6 per cent. ; with an intake of 357 to 588 grams, in the shape of peas, 3·6 to 7·0 per cent. was unabsorbed ; of 718 grams of potato 7·6 per cent. was not utilised, but in no case was glycosuria produced. Hence when a meal of starchy food, of whatever amount, causes sugar to appear in the urine, it may be concluded that there is some definite morbid cause at work, and that the individual is, potentially at least, a diabetic. This differentiation of alimentary glycosuria *ex amylo*, from alimentary glycosuria *e saccharo* was first suggested by Naunyn, and is now held by most observers, an inability to assimilate a moderate amount of starchy food without glycosuria resulting, being generally taken as a criterion of pathological glycosuria, and furnishing the basis for the modern dietetic treatment of diabetes, as we shall see later.

After the ingestion of an excess of dextrose the same sugar is

found in the urine, but Strauss and others have in addition met with a levo-rotatory reducing substance, which P. Mayer regards as glucuronic acid. According to v. Noorden, although some levulose is excreted, after an excess of that sugar has been taken, it is chiefly dextrose that appears in the urine. With cane-sugar, and lactose, any excess that is may be absorbed unhydrolised from the intestine into the lymph and blood, reappears in the urine as such, for the unhydrolised sugars cannot be utilised by the tissues. Of the disaccharides, maltose alone is said to be split up in the blood, as the latter normally contains the ferment " maltase." It is stated by v. Noorden that the assimilation limit of some otherwise healthy persons is particularly low for this sugar, and that as little as half a litre of beer, which is the only common food material containing much maltose, is sufficient to cause sugar to appear in the urine. The explanation he offers is that in these individuals the maltose-splitting ferment of the blood is deficient.

Von Noorden has shown that if the assimilation limit for dextrose is gradually exceeded, during successive experiments on the same person, the whole excess is not excreted in its entirety, but that only a certain proportion, varying with the individual, appears in the urine. In two such series of experiments the following results were obtained :—

A

After an intake of 100	grams of dextrose	0·00	gram in the urine
,,	,,	150	,,	,,	0·15	,,	,,
,,	,,	180	,,	,,	0·25	,,	,,
,,	,,	200	,,	,,	0·26	,,	,,
,,	,,	250	,,	,,	0·52	,,	,,

B

After an intake of 100	grams of dextrose	0·00	gram in the urine
,,	,,	150	,,	,,	0·00	,,	,,
,,	,,	180	,,	,,	0·25	,,	,,
,,	,,	200	,,	,,	0·71	,,	,,
,,	,,	250	,,	,,	0·64	,,	,,

In any case the total sugar excreted is seldom more than 2 per cent., or even at the highest, 3 per cent. of the intake. Raphael, however, observes the paradoxical fact that some persons appear to deal more efficiently with large than with small doses.

Injected directly into the circulation, dextrose and levulose, even in large amounts, can be assimilated, provided that the injection is conducted slowly. If the injection is too rapid, or too large a dose is given (more than one gram per kilo of the body-weight),

glycosuria results. Glucose and levulose appear in the urine almost exclusively, but a fraction is said to be modified during its stay in the body, and is excreted as glucuronic acid (Lépine). If elimination by the kidneys is prevented, by tying the ureters, the excess of sugar undergoes changes which give rise to the formation of lactic acid, aceto-acetic acid, acetone, and other substances, with resulting convulsions, coma, and death. Starch in solution, cane-sugar, lactose, and maltose are not assimilated when injected into the blood-stream, but are at once excreted in the urine. After the injection of one gram per kilo, Pavy recovered from the urine passed in one hour :—

Saccharose.	Maltose.	Lactose.	Galactose.	Levulose.	Glucose.
81	56·5	48·7	28·9	20·9	15·6

The subcutaneous injection of as much as 69 grams of dextrose only gives rise to traces of sugar in the urine, but with 100 grams Voit found that 2·6 grams were excreted in the following seven hours. Levulose appears to give much the same results, although experiments with this sugar are few. Voit found traces in the urine after injecting 31 grams. The injection of 30 grams of galactose was followed by the appearance of traces of a reducing sugar in the urine, but 28 grams of maltose did not give rise to any glycosuria. Similar doses of saccharoses, and lactose, were found to give rise to glycosuria, the sugars reappearing in the urine unchanged. The injection of as little as 10 grams of sorbose was followed by the appearance of 3·7 grams (36 per cent.) in the urine.

The differences in the assimilation limit shown by the organism for various sugars is thus not only dependent upon their chemical composition, but also upon their configuration, a character which we have seen to be of great importance in determining the attack of yeasts and micro-organisms on the sugars. Fermentation is but a special instance of a general law, that the space disposition of the chemical components of the bioplasm of a living cell must bear a certain well-defined relation to that of the food material offered to it, before the latter can be utilised for the production of energy and for growth. It is a law that applies to the highest as well as to the lowest organisms, and is of the utmost importance in the physiology and pathology of assimilation. The first example of such a relation between configuration and assimilation was demonstrated by Pasteur, who showed that *Penicillium glaucum*, *Aspergillus niger*, and other moulds, when grown in a solution of inactive racemic-tartaric acid, destroyed the levo-rotatory acid, and left the dextro-rotatory variety, which could thus be recovered.

Some bacteria have been found to attack dextro-rotatory, others levo-rotatory, lactic acid, while sorbose bacteria develop only in alcohols of a definite composition and space configuration, oxidising such substances as glycerine, erythrite, l-arabinose, d-sorbite, d-mannitol, &c., to the corresponding ketones ; but glycerol, l-xylite, dulcite, &c., are unaffected. The oxidation of the aldehyde sugars by bacteria to the corresponding acids is in like manner found to depend upon their configuration.

A similar relation between oxidation and configuration exists in the higher organisms, even in warm-blooded animals. Thus it has been shown by Brion that if the sterioisomeric tartaric acids were given to a dog with its food, in quantities of about 0·2 to 0·75 gram per kilo of its body-weight, the following proportions were recovered from the urine :—

Racemic-tartaric acid	.	.	.	25·0 to 42·0 per cent.
Dextro-	,,	,,	.	.	. 25·0 ,, 29·0	,,
Levo-	,,	,,	.	.	. 2·7 ,, 6·4	,,
Meso-	,,	,,	.	.	. 2·4 ,, 6·7	,,

From this it would appear that the racemic- and dextro-acids are broken down with difficulty in the organism, but that the levo- and meso-varieties are more easily assimilated.

Experiment has shown that a previously sugar-free dog only begins to pass sugar in its urine when it consumes 1·9 to 2·5 grams of grape-sugar per kilo of its body-weight, but that when galactose is substituted, sugar appears in the urine if 0·2 to 0·4 gram per kilo are taken (Hofmeister). Mannose appears to be even less easily assimilated than galactose. Rosenfeld found that 20 grams of dextrose caused no glycosuria in a dog of 7 kilos, and that the same amount of galactose caused 3·2 grams of sugar to appear in the urine, while after 20 grams of mannose 4·2 grams of sugar were excreted. Further experiment showed that l-mannose was assimilated with greater difficulty than d-mannose. Neuberg and Wohlgemuth administered the sterioisomeric arabinoses by the mouth to rabbits, and recovered from the urine a proportion which varied with the conformation of the sugar molecule :—

l-arabinose	.	.	.	. 14·49 per cent. (l-)
d-arabinose	.	.	.	. 39·97 ,, (d-)
r-arabinose	.	.	.	·$\begin{cases} 21·59 & ,, & (r-) \\ 9·0 & ,, & (d-) \end{cases}$

With subcutaneous and intravenous injections very similar readings were obtained. It would therefore seem that l-arabinose is much more easily assimilated than d-arabinose, and that the

racemic variety is partly decomposed, the l-portion being oxidised, while part of the d-portion is secreted in the urine unchanged. Only one sugar with more than six carbon atoms, a-glycoheptose, has been investigated, and this Wohlgemuth found was assimilated with much greater difficulty than the hexoses.

The above, and numerous other experiments on similar lines, have demonstrated that the assimilation, like the fermentation, of sugars is dependent upon their structure and configuration, and that, although there are individual variations, each sugar has an assimilation limit which is fairly constant for healthy persons.

Pathological Alimentary Glycosuria

The assimilation limit for sugar in disease has been the subject of numerous investigations, and it has been found that it is particularly liable to be lowered by pathological conditions of (1) the nervous system, (2) the liver, (3) the pancreas, thyroid, &c., and (4) as the result of toxic disturbances dependent upon certain infectious diseases, poisons, drugs, &c. For the purpose of such investigations, dextrose, levulose, and cane-sugar have been chiefly employed, but as the absorption of the last named is very liable to be influenced by the condition of the intestinal wall and contents, the results obtained by its use are not so reliable as those found after the administration of the simple monosaccharides.

I. **Alimentary Glucosuria.**—It is customary when testing the dextrose tolerance of an individual to administer a single dose of 100 grams, dissolved in a quarter of a litre of water or tea, in the morning fasting or a couple of hours after breakfast, so that it is taken into an empty stomach. After such a dose a healthy person excretes no sugar in his urine, but if, for any reason, his tolerance is lowered, sugar will be found in about half an hour, and will as a rule persist for three or four hours. Sometimes it happens that sugar will continue to be excreted for several days, a phenomenon which suggests the presence of a serious derangement of carbohydrate metabolism. If 100 grams of dextrose suffices to induce glycosuria smaller quantities should be tried on succeeding days, until an amount is found which is not followed by the appearance of any sugar in the urine. This represents the limit of tolerance for dextrose of the particular case under investigation. In some instances it will be found that 50, 25, or even 10 grams will be sufficient to produce glycosuria, but when the limit is lowered to 10 or 15 grams it will usually prove that the limit of tolerance for starch is also reduced, and the patient is a potential diabetic.

1. *In Nervous Diseases.*—The discovery by C. Bernard that puncture of the floor of the fourth ventricle in animals gave rise to glycosuria, stimulated research into the relation between diseases of the nervous system and diabetes in man. In the course of these investigations the question of alimentary glycosuria in diseases of the nervous system naturally received considerable attention.

Strasser administered 100 grams of dextrose to thirty-seven cases of disease of the central nervous system, and found glycosuria in seven, more often with affections of the brain than of the spinal cord. It was found by Oordt that alimentary glycosuria is more common with cerebral tumours than with other organic diseases of the brain, while Haedke obtained a positive result with fifteen out of twenty-five cases of injury to the skull. Klippel, Vigoroux, and Juquelier state that alimentary glycosuria is most easily provoked in patients with mental confusion, hallucinations, and delirium, and that the tendency disappears as the mental condition improves. Epilepsy, according to Oordt, does not appear to predispose to alimentary glycosuria, and in simple hysteria and hypochondriasis it is not common. Strauss investigated the sugar tolerance of thirty cases of tabes, and only obtained a positive result with one. A long series of cases of nervous disease were investigated by Arndt, with the following results :—

55 cases of General paralysis			5 positive (10 per cent.)			
31	,,	Hysteria	2	,,	(6	,,	)
7	,,	Hypochondriasis	0	,,	(0	,,	)
21	,,	Melancholia	5	,,	(24	,,	)
7	,,	Stupor	1	,,	(14	,,	)
6	,,	Mania	1	,,	(16	,,	)
13	,,	Epilepsy	0	,,	(0	,,	)
11	,,	Traumatic neuropsychoses	4	,,	(36	,,	)

It is evident that in certain conditions of the nervous system the capacity of the body for assimilating glucose is diminished. This appears to be most common in traumatic neuroses, cerebral tumours, acute diseases of the brain and meninges, neurasthenia, and forms of mental debility, particularly mania and paralysis, in which it may be assumed that either the diabetic centre in the medulla is excited, or that the nervous arc, of which it is the centre, is so affected that the liver is unable to store glycogen in any quantity, especially when a large amount of sugar is suddenly thrown into the portal vein.

2. *In Pathological Conditions of the Liver.*—In 1875 Colrat announced that alimentary glycosuria is an important sign of disease of the liver, and particularly of cirrhosis, pylephlebitis,

and other conditions, in which the portal vein is more or less obstructed. His conclusions were confirmed by Baylac, who stated that a positive result was always obtained in such cases, excepting when absorption was interfered with. Campagnolle, and Niepraschk, came to the same conclusion, but found that there was no relation between the alimentary glycosuria and the intensity of the liver disease. De Haan, who examined twenty-nine cases, obtained a positive result with eighteen (62 per cent.).

The value of these, and many other observations, mostly by members of the French school, is discounted, however, by the fact that cane-sugar was generally used for the tests. Strauss, who employed dextrose, only obtained a positive result with one out of twenty cases of liver disease, in marked contrast to the French observers. Valmont failed to obtain alimentary glycosuria with a single one of the seven cases of cirrhosis of the liver that he examined, and Krauss and Ludwig only obtained a positive result with three out of seven cases, and in two of these the dose of sugar exceeded 100 grams. Bloch examined nine cases of liver disease, mostly cirrhotic, with regard to their tolerance for sugar, and found that the urine of only one gave any reduction, although several others were levo-rotatory, probably from the presence of glucuronic acid. Other observers have also reported negative results with cirrhosis of the liver. Three cases of catarrhal jaundice were investigated by Zülzer with negative results. The same observer found that cholelythiasis and amyloid disease of the liver did not predispose to alimentary glycosuria. After the administration of 100 grams of dextrose v. Jaksch recovered from the urine of a case of acute phosphorus poisoning 20 grams of sugar, and Walko subsequently reported several similar cases. The presence of alimentary dextrosuria has been recorded by v. Noorden in cases of fatty liver.

It may be concluded that, although alimentary dextrosuria can be frequently produced in cases where there is disease of the liver, by the administration of 100 grams or more of glucose, it has not the diagnostic significance that it was at one time supposed to have.

3 (a). *In Diseases of the Pancreas.*—The relation of the pancreas to alimentary dextrosuria in man was investigated by Wille, who gave 70 to 100 grams of grape-sugar to eight hundred patients, suffering from a variety of diseases, in the morning before food had been taken. Their urine was examined before the test, and at intervals of two hours afterwards. Of these eight hundred individuals seventy-seven subsequently died, and were examined post-

mortem. Alimentary glycosuria had been found in fifteen. In ten (65 per cent.) of them there were grave lesions of the pancreas, either primary or secondary to growths of the stomach, liver, or gall-bladder. Alimentary dextrosuria has also been reported by Niepraschk in association with cancer of the pancreas, and by Kraus and Ludwig with a cyst of the pancreas.

Pratt and Spencer found that the rapid atrophy of the pancreas produced in dogs by satisfactory and complete ligature of the pancreatic ducts, quickly lowers their assimilation limit for dextrose. In one dog it fell from 121 grams to 65 grams within three weeks, and in two others it dropped to 35 grams in from two to three months.

(b) *In Diseases of the Thyroid Gland.*—Arndt discovered that alimentary dextrosuria is favoured by the presence of exophthalmic goitre, and Bettmann showed that a sufficiently prolonged administration of thyroid preparations by the mouth produces a similar result. On the other hand, Knopfelmacher states that in myxœdema the power of assimilating sugar is remarkably increased, and that this is notably diminished after treatment with thyroid preparations.

(c) *Influence of the Kidneys.*—Other things being equal, alimentary dextrosuria is favoured by the administration of a diuretic (Gobbi), and is less liable to occur when the permeability of the kidneys is diminished from any cause (Achard and Castaigne).

4. *Influence of Toxines*—(a) *Alcohol.*—Small doses of alcohol do not notably influence the assimilative powers for dextrose, and chronic alcoholism does not predispose to alimentary dextrosuria as much as might be expected. Strauss obtained a positive result with 7 per cent. of the cases he examined, and Arndt with three out of twenty-three cases, two of which had also been exposed to the influence of lead. Acute alcoholism, on the contrary, gives rise to alimentary dextrosuria in a considerable proportion of cases. Strauss found that it was present in 70 per cent. of the cases of delirium tremens that he investigated, and Arndt obtained very similar results, thirteen out of twenty-nine cases (65 per cent.) of acute alcoholism in his experience having alimentary glycosuria.

(b) *Lead.*—Acute and chronic lead-poisoning, but more particularly the former, are said to give rise to alimentary dextrosuria (Strauss). A positive result was obtained by Rosenberg in 60 per cent. of cases, mostly with colic. Since, according to Mosse, lead is deposited in the cœliac plexus in experimental lead colic, and extirpation of the plexus gives rise to glycosuria, it is probable

that the tendency to glycosuria seen in such cases is dependent upon disease of the cœliac plexus.

(c) *Carbon Monoxide.*—We have seen that spontaneous glycosuria is not uncommon as a result of poisoning with carbon monoxide. Even in those cases where this does not occur alimentary glycosuria is easily induced, according to Muenzer and Palma.

(d) *Other toxic substances,* such as chloralhydrate, copaiba. nitro-benzol, &c., have been said to give rise to alimentary glycosuria on the results of the reduction tests ; but the reduction in these cases is more probably due to glucuronic acid, as the presence of sugar cannot be confirmed by the fermentation and phenylhydrazin tests.

(e) *Febrile Diseases.*—According to Poll, febrile diseases are frequently associated with a lowered tolerance for dextrose, from 0·45 to 8·0 per cent. of the administered sugar reappearing in the urine in the cases of pneumonia, typhoid, scarlet fever, acute articular rheumatism, &c., that he investigated. Campagnolle confirms these results, but Blumenthal found that the reduction, in many cases at least, is due to glucuronic acid.

(f) *Syphilis.*—Paris and Dobrovici observed alimentary dextrosuria in four out of ten cases of syphilis, but their results lack confirmation.

(g) *In other diseases* alimentary dextrosuria has also been described by a few observers. Nobécourt obtained a positive result with seven out of twelve rickety children. Nagelschmidt examined seventeen cases of psoriasis, and found that the urines of five gave a reduction. Naunyn states that chlorosis predisposes to alimentary glycosuria, while Mayer and Pick observed the same with regard to obesity, obtaining a positive result with twenty out of fifty cases they examined.

II. **Pathological Alimentary Levulosuria.**—This condition has been chiefly investigated with regard to the influence of diseases of the liver, and its presence is regarded by most authorities as evidence of hepatic insufficiency. In 1899 Sachs, working under Strauss' direction, found that after extirpation of the liver in frogs there was a lessened tolerance for levulose when it was injected into the lymph sac, but not for dextrose, galactose, or arabinose. In a later paper he stated that the liver was able to form glycogen from levulose, but that the muscles had not this property, whence it might be assumed that alteration in the functions of the liver would be shown by a diminished tolerance for levulose. Clinical experience tended to confirm this conclu-

sion, for he found that, whereas healthy individuals could assimilate levulose better than dextrose, tolerance for the same amount of levulose was much diminished in persons with liver diseases. Strauss investigated the tolerance for levulose of eighty-seven persons, and found that, of twenty-nine who were suffering from disease of the liver, twenty-six (90 per cent.) showed alimentary levulosuria, while of fifty-eight who had no obvious liver trouble, only six (10 per cent.) passed levulose in their urine ; and of these six two were drunkards and suffered from obesity and gout, one had some hepatic congestion secondary to mitral stenosis, one was suffering from anæmia due to gastric hæmorrhage, and one had pneumonia, in which, according to Rosenberger, levulosuria is frequently met with. Of the cases of hepatic disease that did not show levulosuria, one had atrophic cirrhosis and severe diarrhœa, causing interference with the absorption of the sugar, one had acute cholecystitis of two days' duration, and a third had a cyst of the liver.

Results exactly corresponding to those of Strauss were obtained by Bruining, who examined eleven cases of cirrhosis of the liver, and found that ten (90 per cent.) showed alimentary levulosuria. Baylac and Arnaud reported a positive result with twenty-one out of twenty-three cases (91 per cent.) of liver disease that they examined, but observed alimentary levulosuria in a much higher proportion of cases presenting no symptoms referable to the liver than Strauss, seven out of twenty (35 per cent.) passing sugar in their urine after 100 grams of levulose. With regard to the latter they point out, however, that slight derangements of the hepatic functions are not uncommon in hospital patients such as they examined. Landsberg was unable to confirm the diagnostic value of the test, and considered that idiosyncrasy is more important in producing alimentary levulosuria than functional disease of the liver. He obtained a positive result with four out of seven healthy persons, but with only nine out of twenty-one cases (43 per cent.) of patients with affections of the liver that he examined, including four out of eleven with cirrhosis, one out of four with carcinoma, two with hypertrophic cirrhosis, and two with chronic obstruction of the bile duct, while one with icterus and biliary calculi, and one of congested liver, showed no alimentary levulosuria. He suggests that the higher percentage of positive results recorded by Strauss is to be attributed to his taking only advanced cases in which a collateral circulation had been established, thus allowing rapid entrance of levulose into the blood and urine. In 1904 Chajes collected all the cases of alimentary levulosuria reported up to that date, and added twenty-one observations on normal individuals, showing

only one positive result, of his own. Of eighty-four cases of clinical liver disease he found that fifty-two (86 per cent.) showed alimentary levulosuria, while of ninety-nine persons whose liver was supposed to be healthy only 15 per cent. passed sugar in their urine as a result of the test.

Von Halász considers the test of great service in deciding between cirrhosis and other conditions. He states that normally 100 grams of levulose rarely cause levulosuria, and that a positive result points to diffuse and severe disease of the liver, and is especially indicative of cirrhosis. Goodman, who investigated thirty-two cases, came to the conclusion that, while not indicative of any specific organic lesion of the liver, alimentary levulosuria is most frequently observed in cirrhosis, with which it is almost a constant phenomenon, and that the early, or late, appearance of levulose in the urine may be regarded as a sign of severe, or mild, hepatic disease. He obtained a positive result with all the twenty cases of cirrhosis he tested, a speedy appearance of sugar coinciding with severe disorders, and a tardy appearance with clinically mild affections. Six cases with chronic passive congestion showed no levulosuria, and Goodman points out the usefulness of this test as a means of diagnosing that condition from cirrhosis. A positive reaction was obtained in 12·5 per cent. of non-hepatic diseases.

Hohlweg administered 100 grams of levulose, dissolved in 300 c.c. of water, fasting, to forty-one cases of various liver affections, and states that the tolerance was most reduced with cirrhosis, catarrhal jaundice, and obstructions of the common duct by gall-stones. A stone in the cystic duct, or tumour of the liver, did not give rise to alimentary levulosuria. The different effects produced when the bile duct was obstructed by a tumour, and by a gall-stone, were found to be very striking. He came to the conclusion that all affections that injure the parenchyma, reveal the functional disturbance by alimentary levulosuria, but that the assimilation is scarcely interfered with in cases of enlargement of the liver, leuchæmia, and congestion, or tumours. He confirmed these clinical findings by the results of experiments in which pathological conditions of the liver were induced by injections of toluilendiamin, phosphorus, or a mixture of chloroform, oil, and paraffin. Sabatowski applied the test in seventy-eight cases of liver disease. Positive findings were constantly obtained with cirrhosis of the liver, and were also the rule in infectious diseases, and jaundice of infectious and toxic origin. Jaundice from obstruction did not give rise to alimentary levulosuria unless there were anatomical changes in the parenchyma, and nutmeg

liver also gave a negative result, excepting when the parenchyma was much damaged. He believes that alimentary levulosuria is independent of stasis of bile, but is a constant accompaniment of every severe liver affection, and may serve to differentiate an infectious process in the liver. Frey found that alimentary levulosuria occurred in 10 per cent. of persons with healthy livers, notably in association with affections of the pituitary gland, and in 59 per cent. of those in which that organ was diseased. He states that a positive result is especially frequent when there is cirrhosis, but does not constantly accompany that condition.

The relation of alimentary levulosuria to diseases of the liver in children was investigated by Brun. He gave various doses of levulose to four hundred sick and about one hundred healthy children, ranging in age from one month to twelve years, and found that "it was an easy, delicate, and harmless test for deter-mining the functional capacity of the liver. It reveals the slightest impairment of function, and also the progress toward recovery or the reverse." He found that the normal liver does not permit levulose to pass unmodified.

III. **Pathological Alimentary Galactosuria.**—Comparatively few researches into the influence of disease upon the excretion of galactose have been carried out. According to Bauer the ad-ministration of 29 grams of this sugar, fasting, which does not cause the appearance of sugar in the urine in health, is followed by galactosuria in patients suffering from organic, or functional, dis-orders of the liver. One hundred grams cause a more marked excretion, but also gives rise to galactosuria and dextrosuria in the healthy. It is claimed by Bauer and others that the assimi-lative capacity of the body for milk-sugar when 40 grams are given in the morning after free purgation, gives more satisfactory indications of the functional capacity of the liver than levulose, and that it is also more readily taken. Normally only 0 to 1 gram of sugar can be detected in the urine subsequently, and this appears, if at all, in the first hour, and all traces having disappeared by the fourth hour. If, however, the liver cells are diseased, 4 to 10 grams of galactose are eliminated by the kidneys, the largest amount appearing in from three to six hours and continuing for ten to fourteen hours. The test was originally based on the observation by Bauer that a man with Hanot's cirrhosis who took large quan-tities of milk passed an unfermentable sugar in his urine, and that the excretion ceased when the milk was stopped.

IV. **Pathological Alimentary Lactosuria.**—Lactosuria has

been observed by v. Halász to follow the ingestion of a moderate amount of milk in patients with dilated stomach. It has also been stated by Grosz that an excess of milk sometimes causes a reducing substance, having the reactions of lactose, to appear in the urines of infants, particularly when they are suffering from gastro-intestinal catarrh.

Zülzer investigated alimentary lactosuria during the puerperium, and found that 60 grams of lactose did not cause sugar to appear in the urine of any of the cases he examined, but that after 100 grams, eleven out of thirteen cases gave a slight reaction. Lactosuria was only exceptionally met with after a similar dose in normal women.

Von Noorden gave 159 grams of cane-sugar to lying-in women, after abortion or premature labour, and recovered milk-sugar from their urine.

V. **Pathological Alimentary Maltosuria.** —I am not acquainted with any observations on the effects of disease in reducing the tolerance for maltose, but, judging from the readiness with which comparatively small quantities of beer sometimes produce maltosuria, it is not unlikely the tendency may be increased by various pathological conditions.

VI. **Pathological Alimentary Saccharosuria.**—Many of the original observations on sugar tolerance were made with canesugar, and although glycosuria was found to follow its ingestion in cirrhosis of the liver, and other conditions giving rise to hepatic insufficiency, by some observers, others obtained conflicting results. It was not until pure levulose and dextrose were employed that the more constant findings already referred to were secured. There can be no doubt that the occurrence of glycosuria after the administration of saccharose is largely controlled by the condition of the alimentary tract, and for this reason the simple monosaccharides are to be preferred for experimental investigations. Traces of cane-sugar are said to have been found in the urine in children after a considerable quantity has been consumed, but as a rule it appears in the form of dextrose, possibly accompanied by some levulose.

VII. **Pathological Alimentary Pentosuria.**—Nothing is known of the effects of disease on the production of alimentary pentosuria. According to v. Jaksch, large doses of arabinose, xylose, and rhamnose give rise to diarrhœa, and the sugars can be recovered from the urine, a quarter to a half of the quantity ingested being sometimes thus excreted. I have found that in

a healthy man the ingestion of 0·5 gram of xylose was followed by the appearance of 0·08 gram of that sugar in the urine within twenty-four hours.

TRANSITORY AND INTERMITTENT GLYCOSURIA

The transitory forms of glycosuria already considered, whether in health or disease, are produced artificially by the administration of a relatively large dose of sugar under experimental conditions, but spontaneous or idiopathic transitory glycosuria is also known to occur. This is met with as an epiphenomenon in the course of various organic, or constitutional, diseases. It appears without the addition of any extraordinary amount of sugar, or other carbohydrate, to the diet, and disappears again as the condition with which it is associated improves. The existence of sugar in the urine in such cases is usually discovered accidentally in the course of a routine examination, and its presence is not associated with any characteristic symptoms.

I. *Transitory Glycosuria*

(*a*) *In Nervous Diseases.*—Such a transitory glycosuria, apparently of central origin, has been noticed in connection with lesions of both the central and peripheral nervous system, such as tumours and hæmorrhages at the base of the brain, lesions of the floor of the fourth ventricle, cerebral and spinal meningitis, concussion of the brain, fracture of the cerviacal vertebræ, tetanus, and sciatica. It has also been met with after epileptic, hystero-epileptic, and apoplectic seizures, in traumatic neuroses, such as those following railway accidents, mental shocks, mental strain, worry, fatigue, and great anxiety. Glycosuria following mental strain and anxiety is not at all uncommon in business and professional men, but if the cause of the condition is recognised at a sufficiently early stage, and the patient is placed under satisfactory hygienic conditions, both physical and mental, the sugar generally disappears as the general health improves. In several such patients I have been unable to induce even alimentary glycosuria, by the administration of 100 grams of dextrose or levulose, taken fasting, after a sea-voyage, or other form of " rest-cure." One case has been under observation for five years, and another for three, without any return of the glycosuria, but it is as yet too early to say that this may not occur.

Transitory glycosuria was observed by Siegmund in 52 per cent.

of cases of general paralysis, in 7·4 per cent. of epileptics, and in 3·77 per cent. of dementia cases, but he did not meet with it in other mental diseases. Glycosuria after epileptic and apoplectic seizures does not, however, appear to be as common as it is frequently stated to be. Von Jaksch examined fifty recent cases of hemiplegia and did not find sugar in the urine in a single instance, while Simon had a similar experience with the urines of a large number of epileptics examined within a few hours of the attack.

Kausch has reported eleven cases of recent injury, nine of fracture and two of contusion, in which glycosuria was present. He states that the sugar found was in minute quantities, the maximum being 1 per cent. and the average 0·5 per cent. It appeared almost immediately after the injury, being present in the first specimens of urine examined. The glycosuria disappeared in all cases in the course of a week, usually about the third day, and was never associated with any other diabetic symptoms. With one exception all the patients remained in a normal condition with regard to their urine. According to Ott, the transitory glycosuria that occurs during pregnancy, in nervous cases, and ends after delivery, is very probably due to hyper-hypophsy, although the promotion of activity of the chromaffin tissue by the thyroid is most likely also contributory.

(b) *Infectious Diseases.*—We have seen that alimentary glycosuria is favoured by the presence of infectious diseases, and it is therefore not surprising to find that spontaneous transitory glycosuria is occasionally observed in acute febrile disorders, and more particularly during convalescence from them.

The existence of a reducing substance in the urine of patients suffering from malaria was pointed out by Burdel, who obtained a positive result with 17 per cent. of non-cachectic, and 76 per cent. of cachectic cases. Calmette examined forty-one soldiers suffering from malaria and found glycosuria in five, about the same proportion as in Burdel's non-cachectic cases. Seegen reported five cases of malaria with " diabetes," in which both conditions disappeared under the administration of quinine. Although the glycosuria in this instance may be referred to the action of toxines, it would seem that the general condition of the patient has more influence on its production than the intensity of the intoxication. It is possibly akin to the *vagabond glycosuria*, described by Hoppe-Seyler, who found that tramps who had walked long distances on a diet of bread and potatoes frequently passed urine containing 0·5 to 0·7 per cent. of glucose on admission to an institution. The sugar quickly disappeared, even on a diet rich in carbohydrates,

and did not return, although large doses of dextrose were given on an empty stomach.

Glycosuria appears to be not uncommon in diphtheria. Binet obtained a positive result in twenty-nine out of seventy cases. Of these thirty-eight were severe, with twenty-seven positive results, and three mild infections, with two positive results. Simon met with a transitory glycosuria in four out of thirty-two cases of the same disease of moderate severity. Hibbard and Morrissey noticed the existence of a glycosuria, of variable duration, and frequently associated with albuminuria, in serious cases of diphtheria, and have also pointed out that the injection of anti-diphtheritic serum is sometimes followed by the temporary appearance of sugar in the urine.

In one case Teschemacher reports that the injection of Koch's tuberculin was followed by temporary glycosuria.

Transitory glycosuria has also been met with in typhoid fever, scarlet fever, measles, cholera, influenza, whooping cough, pneumonia, smallpox, and other infectious diseases, including syphilis. Manchot obtained a reduction with the urines of twelve out of 359 patients suffering from the last-mentioned disease in the second stage.

(c) *Toxic Conditions*, &c.—The absorption of toxic materials affecting the nervous system, or possibly the pancreas, may account for the transitory glycosuria noticed by Hoppe-Seyler in cachectic individuals with intestinal troubles, and in dyspeptics by Robin. While a similar explanation possibly holds good for the sugar found in the urine of children affected with tape-worms by Judson, and with round worms by Parry. Glycosuria has also been noticed in association with cysticircus tumours by Marie and Guillain and others, but it is not improbable that this was merely a coincidence.

(d) *Surgical Conditions*.—Transitory glycosuria has been reported in various surgical affections. Leidy met with it in two cases of appendicitis, and Cohen has reported a similar case, in which, after deferring operation because of the sugar in the urine, it was found to disappear two days after the appendicular abscess had been opened. Cohen suggests that the glycosuria was due to irritation of the solar plexus. Malcolm has met with glycosuria in association with an ovarian cyst, which disappeared on removing the tumour. Robinson has reported what appeared to be levulosuria, during an attack of gonorrhœa, that disappeared when the discharge was cured. As long ago as 1861, Hill described glycosuria in four cases of severe burns, and, according to Vannini, it is not an uncommon complication.

The occurrence of transitory glycosuria in connection with the foregoing diseases, while startling at the time, is usually of no diagnostic or prognostic importance. It is advisable that the urine should be watched and examined at intervals for sugar, since occasionally a persistent glycosuria may subsequently make its appearance, but as a rule the condition is a temporary one, and does not indicate any profound or lasting interference with carbohydrate metabolism.

II. *Intermittent Glycosuria*

In addition to the preceding type of case, in which, after the urine has contained sugar for a shorter or longer period, tolerance for carbohydrates becomes completely restored, we have a second group, in which the limit of tolerance for carbohydrates is permanently depressed, and glycosuria occurs whenever more than a certain amount of sugar, or starchy food, is taken, or an intercurrent complication temporarily lowers the patient's assimilative powers. To this class belong the cases of alimentary glycosuria *ex amylo* previously referred to. At present we have no means of distinguishing the truly transitory from the intermittent variety of glycosuria, excepting experience and continued observation ; but as the latter is much more common, and infinitely more serious than the former, it is well to regard every case with sugar in the urine as a possible diabetic and treat it accordingly, unless there is only a trace and the glycosuria speedily clears up after the condition with which it was associated has disappeared.

Intermittent glycosuria may probably be caused by any pathological state that can bring about a persistent excretion of sugar in the urine, but practically nothing is known of any others than disease of the pancreas. Both experiments upon animals, and clinical experience, have shown that the sugar tolerance of patients suffering from chronic inflammatory conditions of the pancreas is lowered in many cases, and it is also known that persistent glycosuria ultimately develops when the cirrhosis of the gland reaches a certain stage. If, therefore, a patient whose pancreas is partly incapacitated as the result of inflammation, exceeds his lowered limit of tolerance, or has it temporarily reduced still further by an attack of subacute pancreatitis, sugar will appear in the urine, to again disappear when the exciting cause is removed. I have had several cases under my observation in which this course of events apparently occurred.

In September 1908 I was consulted by a gentleman of sixty-two,

who had symptoms suggesting the presence of floating gall-stones in the common bile duct. An analysis of his urine and fæces tended to confirm this diagnosis. Operation was advised but refused. In May 1909 I again saw him and found that his urine, which had previously been sugar-free, contained 19 grams (1·2 per cent.) of dextrose. Under treatment this was reduced to 9 grams (0·8 per cent.), and by the end of June it had quite disappeared. He was placed on a restricted diet, and his urine examined at frequent intervals, but it was always found to be free from sugar during the following three months. In July 1911 he returned, and his urine was now found to contain 108 grams (6 per cent.) of dextrose, and his fæces showed a much higher proportion of unsaponified fat, besides giving a well-marked pancreatic insufficiency test (Gross). With careful dieting the amount of sugar in the urine was reduced to 70 grams in the twenty-four hours (4 per cent.), but it could not be removed altogether.

Another patient, who was sent to me in January 1908, had been examined for life insurance, and passed, six months previously. After a long drive in an open carriage, he complained of pain in the back and under the left shoulder, and his medical man on examining his urine found a specific gravity of 1·032 and 2·8 per cent. of sugar. A twenty-four hour sample of the urine that I examined showed 48 grams (3 per cent.) of dextrose, and a trace of acetone, but no aceto-acetic acid. The excretion of ammonia nitrogen was not excessive (0·6 grams). His fæces were acid in reaction, and contained 55 per cent. of unabsorbed fat, including 41 per cent. in the unsaponified form. Casein digestion was found to be imperfect. On physical examination tenderness, and some fulness, in the region of the head of the pancreas could be made out. He was dieted, and treated with pancreatic extracts, urotropine, salicylates, &c., with a view to quieting the pancreatic inflammation, and relieving the gland of as much work as possible. All the symptoms disappeared, and the urine became sugar-free in about six weeks. He remained in good health for two years, and then complained of being easily tired. An examination of his urine showed 28 grams of sugar in the twenty-four hours, a well-marked reaction for acetone and aceto-acetic acid, and 1·6 grams of ammonia nitrogen. The glycosuria and acidosis were reduced by treatment, but his urine could not be again made quite sugar-free.

A case of considerable interest in this connection has been described by Garrod.

The patient, a man aged fifty-eight, came under observation in October 1909, suffering from a moderate degree of jaundice. His urine contained 2·8 per cent. of dextrose. By diet the sugar was reduced in amount, and on December 31, 1909, had quite disappeared. Repeated examinations were made, but the urine remained sugar-free for over two years, although he had discarded all dietetic restrictions, and took some 14 ounces of bread a day, in addition to other starchy food. Even with 100 grams of dextrose, taken fasting, no glycosuria

resulted. Subsequently it was found that the patient was again excreting sugar, but in very small amount, for although a specimen passed at 1.30 P.M. reduced Fehling's solution, and gave an osazone melting at 206° C., specimens taken in the early morning, and at 2.30 P.M., gave no reduction.

In these cases it would seem probable that the temporary glycosuria, and in Garrod's case the jaundice also, was due to a subacute exacerbation of a chronic pancreatitis, which slowly progressed, and ultimately so interfered with carbohydrate metabolism as to induce the constant presence of sugar in the urine.

Hirschfeld has recently reported cases of diabetes which developed after some infectious disease. The diabetes was accompanied by gastrointestinal symptoms, but there was no pronounced polyuria, although the glycosuria lasted for several months. Another special feature of the cases was the accompanying enlargement of the liver, which gradually subsided completely. In a still more recent case the onset of the diabetes occurred six weeks after a severe infectious sore throat ; there was also enlargement of the liver in this case. In another case, a man of forty-six presented symptoms of diabetes such as accompany a transient affection of the pancreas. He had had previously numerous attacks of influenza. After a concussion, due to jumping from a moving car, the diabetes returned in more severe form and became chronic. These and other experiences have convinced Hirschfeld that infection is an important factor in the origin and aggravation of pancreatitis and diabetes, and that chronic pancreatitis, such as is found so frequently in diabetics, generally has developed on the basis of repeated acute or subacute inflammations. Glycosuria lasting only a few weeks or months corresponds to the acute transient pancreatitis.

Temporary glycosuria following an attack of mumps, probably due to involvement of the pancreas, has been reported by Barbieri, and I have had a similar case. The occurrence of temporary glycosuria and acidosis in a man of fifty-eight, in association with unilateral parotitis, was recently reported by Routh. Harris has described two cases of diabetes in which sugar was first found in the urine shortly after an attack of mumps.

The association of temporary glycosuria with gall-stones is most probably to be referred to the pancreatitis that so commonly accompanies the presence of calculi in the common bile duct. Hochhaus has published a case in which a woman passed 33 grams of sugar in twenty-four hours during an attack of hepatic colic ; the next, and four following days, 4 to 5 grams were excreted ;

but after that the sugar disappeared. It is, however, a comparatively rare complication. Zinn only found sugar twice in the urines of eighty-nine cases of hepatic colic, and Kausch three times in eighty-five cases. In an examination of the urines from 396 cases of biliary calculi, I met with sugar in forty-two (11 per cent.), usually, however, only in traces. Of the forty-two cases in which sugar was found, twenty-seven were operated on, and in eight the urine was found to be sugar-free when it was examined a week or ten days later, but in nineteen sugar was still present. I have not been able to obtain the after-histories of many of these cases, but one at least in which the sugar disappeared after the operation developed a permanent glycosuria a few months later, and ultimately died of diabetic coma. It must not therefore be too readily assumed that the improvement that follows operation, when there is obstruction of the bile and pancreatic ducts, is due to the relief of tension and subsidence of the associated catarrhal inflammation, for the rest in bed and comparative starvation are often important factors in bringing about the disappearance of the glycosuria, which may return when the patient resumes his ordinary diet and mode of life.

An example of the association of temporary glycosuria with acute pancreatitis, in which recovery took place but the patient ultimately became diabetic, has been recorded by Gifford Nash.

The patient was a man of sixty who had suffered for seven years from "bilious attacks," and discomfort at the pit of the stomach. On October 27, 1901, he was seized with sudden pain in the abdomen. There was no jaundice, and the symptoms suggested intestinal obstruction. The urine was increased in amount, and contained on November 5th 8·75 grains of sugar per ounce. Operation was undertaken on November 17th. The pancreas was found to be enlarged, there was fat necrosis in the neighbourhood of the gland, and a large calculus was found in the gall-bladder. Cholecystotomy was performed, and the patient slowly recovered. On December 28th the urine contained 4·5 grains of sugar to the ounce, and, on March 1, 1902, 4·5 grains also, but on May 17th the glycosuria had disappeared. In November 1902 I examined a specimen of the urine and found that the sugar had returned, and that it gave a well-marked "pancreatic" reaction. A second specimen examined in February 1904 gave similar results. It was then stated that the patient was in very good health, and had had no illness since his operation. In January 1906 I made a further examination and found that the urine contained 0·95 per cent. of sugar, and no acetone or aceto-acetic acid, but it still gave a positive pancreatic reaction.

Marwedel has recorded a case of abscess of the pancreas in which sugar was excreted for a few days only.

Intermittent glycosuria has been met with in some cases of
pancreatic calculi, owing probably to a temporary inhibition of
the functions of the pancreas from transient attacks of pancrea-
titis. Thus in a case reported by Lancereaux sugar was found
in the urine with each attack of colic, but in the intervals it was
sugar-free, and in a case recorded by Holzmann sugar was found
in the urine at intervals, although when the patient was under
Minnich's care sometime earlier repeated examinations had given
a negative result.

The temporary appearance of sugar in the urine met with in
some cases of cancer of the pancreas is explicable on similar lines,
the inflammatory reaction attendant on the spread of the growth
causing temporary damage, which subsequently quiets down, and
leaves sufficient unaltered tissue to carry on the work of carbo-
hydrate metabolism. In a case of this description quoted by Oser
there was transient glycosuria for seven months, then eleven
months cachexia without glycosuria. After death, which took
place twenty-three months after the onset of the disease, a large
hard cancerous mass was found occupying the head, and half the
body, of the pancreas, the rest of the gland appeared to be normal.
It has to be borne in mind that where a portion of the pancreas
has been destroyed by growth, or inflammatory changes, the con-
dition resembles that produced in animals by partial extirpation
of the gland, so that, if carbohydrates are excluded from the diet,
an alimentary glycosuria which previously existed may disappear.

BIBLIOGRAPHY

Achard and Castaigne, *Bull. soc. méd. d. hôpit. d. Paris*, 1897 ;
    Semaine méd., 1897 ; *Arch. d. Médicine*, 1898.
Arnat, *Deut. Zeit. f. Nervenkrank*, 1897.
Arndt, *Berl. klin. Woch.*, 1898 ; *Deut. Zeit. f. Nervenheilk.*, 1897,
    1899.
Barbieri, *Gazz. degli Ospedali*, 1909.
Bauer, *Wiener med. Woch.*, 1906–1908.
Baylac, *Arch. f. Verdauungskrank*, iv.
Baylac and Arnold, *Compt. Rend. d. Med.*, Toulouse, 1902.
Bettmann, *Münch. med. Woch.*, 1896.
Binet, quoted Lépine, *Diabète Sucré*, 1909.
Bloch, *Zeit. f. klin. Med.*, 1892.
Blumenthal, *Path. d. Harnes*, 1903.
Bouchard, *Traite d. Physiol. Gen.*, iii.
Brion, *Zeit. f. phys. Chem.*, 1898.
Bruel, *Arch. f. spec. Path. u. Pharm.*, 1898.

Bruining, *Berl. klin. Woch.*, 1902.

Brun, *Riforma Medica*, 1910.

Burdel, *Union Medical*, 1872.

Calamette, *Gaz. hebdoma*, 1882.

Campagnolle, *Deut. Arch. f. klin. Med.*, lx.

Chajes, *Deut. med. Woch.*, 1904.

Cohen, *New York Med. Journ.*, 1894.

Colrat, *Lyon médicale*, 1875.

Frey, *Zeit. f. klin. Med.*, lxxii.

Garrod, *Lancet*, 1912.

Ginsberg, *Arch. f. d. ges. Physiol.*, 1889.

Gobbi, *Il Policlinico*, 1900.

Goodman, *Journ. Amer. Med. Assoc.*, 1909.

Grósz, *Jahrb. f. Kinderheilk.*, 1892.

De Haan, *Arch. f. Verdauungskrank*, 1898.

Haedke, *Deut. med. Woch.*, 1900.

Von Halász, *Wiener klin. Woch.*, 1908 ; *Deut. med. Woch*, 1908.

Harris, *Boston Med. and Surg. Journ.*, 1899.

Hibbard and Morrissey, *Journ. of Exp. Med.*, 1899.

Hill, *Arch. of Med.*, 1861.

Hirschfeld, *Deut. med. Woch.*, xxxv.

Hochhaus, *Deut. med. Woch.*, 1907.

Hofmeister, *Arch. f. exp. Path.*, 1889, 1890.

Hohlweg, *Deut. Arch. f. klin. Med.*, xcvii.

Holzmann, *Münch. med. Woch.*, 1894.

Hoppe-Seyler, *Münch. med. Woch.*, 1900.

Von Jaksch, *Prag. med. Woch.*, 1892, 1895 ; *Zeit. f. Heilkunde*, xx.

Judson, *Lancet*, 1902.

Kausch, *Deut. med. Woch.*, 1899 ; *Zentralb. f. Chir.*, 1904.

Klippel, Vigoroux, and Juquelier, *Arch. f. Neurolog*, 1902.

Knöpfelmacher, *Wiener klin. Woch.*, 1904.

Kraus and Lüdwig, *Wiener klin. Woch.*, 1891.

Lancereaux, *Journ. d. Med. enterne*, 1889.

Landsberg, *Deut. med. Woch.*, 1903.

Leidy, *Medical News*, 1894.

Lépine, *Diabète Sucré*, 1909.

Manchot, *Monats. f. prakt. Dermatol*, 1898.

Marie and Guillain, *Soc. méd. d. hôpitaux d. Paris*, 1901.

Marwedel, *Münch med. Woch.*, 1901.

Mayer, *Berl. klin. Woch.*, 1899 ; *Deut. med. Woch.*, 1901 ; *Zeit. f. klin. Med.*, 1902.

Mayer and Pick, *Berl. klin. Woch.*, 1902.

Moritz, *Arch. f. klin. Med.*, 1890.

Mosse, *Verh. d. Vereins. f. inn. Med.*, 1902.

Muenzer and Palma, *Zeit. f. Heilkunde*, 1894.

Nagelschmidt, *Berl. klin. Woch.*, 1900.

Nash, *Lancet*, 1902.

Neuberg and Wohlgemuth, *Zeit. f. phys. Chem.*, 1902.

Niepraschk, *Inaug. Dissert.*, Berlin, 1898.

Nobécourt, *Compt. Rend. d. l. Soc. de Biol.*, 1900.

Von Noorden, *Zeit. f. klin. Med.*, xxxviii. ; *Arch. f. Anat. u. Phys.*, 1893.

Oordt, *Münch. med. Woch.*, 1898.

Oser, Nothnagel's *Encyclop. of Pract. Med.*, 1903.

Ott, *Internal Secretions*, 1910.

Paris and Dobrovici, *Presse Médicale*, 1892, 1905.

Parry, *Lancet*, 1895.

Pasteur, *Compt. Rend. d. l. Acad. d. Sci.*, 1858, 1860.

Poll, *Fortschr. d. Med.*, 1896.

Pratt and Spooner, *Journ. Amer. Med. Assoc.*, 1910.

Raphael, *Zeit. f. klin. Med.*, 1899.

Robin, *Bull. d. l. Acad. d. Med.*, 1901.

Robinson, *Compt. Rend. d. l. Soc. d. Biol.*, 1899.

Rosenberg, *Inaug. Dissert.*, Berlin, 1897 ; *Centralb. f. inn. Med.*, 1900 ; *Deut. med. Woch.*, 1906.

Routh, *Brit. Med. Journ.*, 1912.

Rubner, *Zeit. f. Biol.*, 1883.

Sabatowski, *Wiener klin. Woch.*, 1908.

Sachs, *Zeit. f. klin. Med.*, 1899, 1900.

Simon, *Clinical Diagnosis*, 1900.

Strasser, *Wiener med. Presse*, 1894.

Strauss, *Berl. klin. Woch.*, 1899 ; *Zeit. f. klin. Med.*, 1900 ; *Deut. med. Woch.*, 1897, 1901.

Teschemacher, *Deut. med. Woch.*, 1895.

Valmont, *Thése de Paris*, 1897.

Vannini, *Rivista critica di Clin. Med.*, 1902.

Voit, *Deut. Arch. f. klin. Med.*, 1897.

Walko, *Zeit. f. Heilkunde*, 1903.

Wille, *Deut. Arch. f. klin. Med.*, 1899.

Wohlgemuth, *Zeit. f. phys. Chem.*, 1902.

Zinn, *Centralb. f. inn. Med.*, 1898.

Zülzer, quoted, Blumenthal *Path. d. Harnes*, 1903.

CHAPTER VI

THE continuous elimination of sugar in the urine is most commonly
one of a complex of symptoms to which the name diabetes mellitus
is applied. In clinical medicine it is usual to distinguish transi-
tory, and intermittent, from persistent glycosuria, and many again
separate the latter from diabetes, but it is important that it should
be clearly recognised that each is but a phase of the same meta-
bolic error, and that no hard and fast line can be drawn between
them. Transitory glycosuria is, as we have seen, evidence that
the metabolic powers of the body are not capable of dealing with
the amount of carbohydrate contained in the food. If the glycos-
uria results from an excessive intake of carbohydrate it is not
necessarily evidence of a pathological state, although the appear-
ance of sugar in the urine after a starchy, as distinguished from a
sugary, diet points to there being defective metabolism, and the
patient must be regarded as a potential diabetic. Should the
glycosuria occur with a normal intake of carbohydrate, it is un-
doubtedly an evidence of disease, which may be permanent, or only
temporary. If the metabolic disturbance is of a transient nature,
and disappears with the removal of the cause, the patient may
be little or none the worse, but if it persists his tolerance for carbo-
hydrate is likely to be still further lowered with the lapse of time,
so that eventually persistent hyperglycæmia and glycosuria will
result. When this condition is established, the natural tendency
is for it to progress, so that eventually the secondary effects of an
excess of sugar in the blood, and the abnormal tissue destruction
that it brings in its train, come into evidence.

The sugar excreted in persistent glycosuria is as a rule dextrose,
but in some cases levulose and other sugars are found in addition.
The quantity of the latter is, however, always relatively small,
and the exact cause and significance of their presence is not under-
stood.

The amount of dextrose that appears in the urine varies very
much, but rarely exceeds 200 grams a day on an ordinary mixed

diet. Occasionally a much larger quantity is met with, as in the case of a diabetic of nineteen reported by Naunyn, who passed 1200 grams of sugar in the twenty-four hours, and in Dickenson's case where 1500 grams were excreted daily. Niedergesäss states that a child of twelve under his care passed 587 grams of sugar in the twenty-four hours, a quantity corresponding to more than 3·8 per cent. of his body-weight. When carbohydrates are excluded from the diet, even the most severe cases rarely pass more than 100 grams a day. In mild cases the sugar excretion increases after food, usually reaching its maximum two or three hours after a meal, and diminishes during fasting, hence the urine passed in the early morning, before breakfast, may be sugar-free. In severe cases the variation is less marked, and more sugar may be excreted during the night than in the day. Muscular exercise reduces the amount of sugar in the urine in well-nourished individuals, and massage has the same effect, but in the more severe forms, where the patient is wasted, as much, or even more, may be excreted after exercise than before. Psychic influences undoubtedly affect the sugar excretion in some cases, the amount being increased by worry, nervous excitement, shock, &c. The output of sugar is usually greater in hot than in cold weather.

Intercurrent affections may influence the glycosuria, some increasing, while others diminish, it. The influence of acute febrile diseases in this respect is very variable, the effect produced appearing to depend on the diet and temperature on the one hand, on the extent of the toxæmia, and consequent interference with the nutrition of the tissues, on the other. Thus in a short sharp infection, such as the cases of follicular tonsilitis described by v. Noorden and Möhr, the sugar is increased, but in more chronic conditions, such as pneumonia and influenza, where the diet is restricted and the temperature high, the sugar may diminish, or even disappear, to return again when convalescence is established. In a similar way the sugar is seen to diminish in some cases when the patient is attacked by enteric fever. But in this, as in other diseases where there is an affection of the gastro-intestinal tract, the interference with food absorption probably influences the sugar excretion in the urine. Of the chronic diseases complicating diabetes the most common is pulmonary tuberculosis, and with this the glycosuria often diminishes, and sometimes entirely ceases a short time before death. When granular kidney is associated with chronic glycosuria the sugar may slowly diminish, and even disappear, leaving only the symptoms of the intercurrent affection. It has been suggested that the disappearance of the sugar in such

cases is due to interference with the excretory functions of the
kidneys, but it was shown by Strauss that the serous effusions
of such cases are not particularly rich in sugar, and according to
Lépine there is no hyperglycæmia, but rather the reverse, so that
it is more likely that the sugar disappears in consequence of the
cachexia that results from the renal changes. According to
Léorché, the excretion of sugar in diabetic women is temporarily
diminished at each menstrual period. A rare but striking cause
of an apparent diminution, or disappearance, of the sugar is the
occurrence of fermentative changes within the bladder. This de-
pends upon infection with yeasts, or fermentative bacteria, which
split up the dextrose into alcohol and carbon dioxide, and, in
addition, give rise to hydrogen, carburetted hydrogen, and other by-
products. Apart from diet and intercurrent affections, the sugar
output is liable to undergo spontaneous variations of considerable
amount, which can only be explained by fluctuations in tolerance.

The volume of the urine passed in persistent dextrosuria is nearly
always increased, varying as a rule with the severity of the case.
Polyuria is generally not marked unless the urine contains 2 to
3 per cent. of sugar. Three or four litres a day is a common amount
to be passed, over 5 litres is rare ; but Naunyn has reported one
case that passed 16 litres, Fürbringer 17 litres, Harnack 18 litres,
and Bence Jones 28 litres. In some instances the amount of urine
excreted does not exceed 1500 c.c. in the twenty-four hours, although
a considerable amount of sugar, 20 to 30 grams for instance, is
present. Such cases have been described by Frank as " diabetes
decipiens." Generally the amount of urine passed rises with an
increase in the output of sugar, but at a slower rate. Sometimes,
however, the volume is augmented without any more sugar being
excreted, and occasionally the reverse occurs. A strict carbo-
hydrate-free diet will reduce the quantity of urine, and it is also
diminished by intercurrent febrile affections, diarrhœa, &c. It
is important to note that several days before the onset of diabetic
coma, and just prior to a fatal termination, a marked reduction
often takes place. In health more urine is excreted as a rule during
the day than during the night, but with most diabetics this dif-
ference is not so marked. In some cases, and particularly those
of a mild type, the night urine is markedly less than the day urine,
but if the condition is complicated by arterio-sclerosis and granular
kidney the reverse condition is often present, especially in the
later stages.

Appearance.—When a urine containing sugar is passed it is
usually bright and clear. It froths more readily than a normal

urine, and the froth is more persistent. If the quantity is not increased, or only slightly, the colour is not altered, but when there is polyuria it is generally of a slight straw colour, and often has a characteristic greenish tint when examined against a white background. On standing, diabetic urine speedily becomes turbid, from the growth of yeasts and fungi. It is often noticed, too, that the " mucous " cloud, instead of forming at the bottom of the vessel as in normal urine, is suspended in the upper layers. A peculiar sweet, or aromatic, odour is frequently observed, and this is particularly noticeable when coma has supervened, or is threatened.

Reaction.—When freshly passed diabetic urine is nearly always acid in reaction, often markedly so, especially in advanced cases, and immediately before the onset of coma. When allowed to stand it remains acid for several days, and may in fact even increase in acidity from the conversion of some of the sugar into lactic acid through the agency of micro-organisms.

Density.—The specific gravity is usually high, ranging between 1·030 and 1·040, but it rarely exceeds 1·050. Bouchard and Prout mention a case that passed a urine with a specific gravity of 1·074. When the specific gravity of a urine is over 1·025, and it is clear and not high-coloured, it is probable that it contains sugar. As a rule, the more sugar there is present the higher is the specific gravity, and vice versâ. As the density does not depend upon the sugar-content alone, but is also influenced by the amount of other solids in solution, the specific gravity cannot, however, be relied upon as an index of the quantity of sugar present. It must be clearly understood that a normal, or even a low, specific gravity does not exclude the presence of sugar, for in some 10 per cent. of cases of persistent glycosuria a sub-normal specific gravity is found, 1·012 to 1·006, or even lower. A low specific gravity is most commonly met with in cases where the absorption of nitrogenous material from the intestine is defective, in the glycosuria following injuries of the head (in which, according to Naunyn, a specific gravity of 1·003 and a sugar-content of about 1 per cent. is often met with), associated with early chronic interstitial nephritis, and when the patient is very weak. In a few cases of diabetes exceedingly low readings have been recorded ; thus in a case reported by Waterman there was a specific gravity of 1·002, and Herrick met with a case in which the density of the urine was only 1·004.

Total Nitrogen.—As we have seen, a healthy individual on an ordinary mixed diet, taking a fair amount of exercise, passes from 10 to 16 grams of nitrogen, with an average of 15 grams, in the twenty-four hours. This comprises by far the greater part of

the nitrogenous loss of the body, less than 1 gram being eliminated through the intestinal secretions and other channels combined. In diabetes 30, 40, or even 50 grams of nitrogen may be excreted in the twenty-four hours. The cause of this increase in the nitrogen excretion, and its relation to the sugar output, will be considered later. Normally the urinary nitrogen is distributed in various compounds as follows : Urea about 86 per cent., ammonia about 3 per cent., creatinin about 3 per cent., uric acid and allied xanthin bases about 2 per cent., the remaining 6 per cent. being furnished in various proportions by substances such as hippuric acid, indol, skatol, &c. The variations in these proportions, and in the total quantities of the nitrogen-containing constituents met with in persistent glycosuria may be summarised as follows :—

Urea.—The urine of diabetics usually contains a sub-normal proportion of urea, but this is due to the polyuria, and, when the total excretion for the whole twenty-four hours is considered, an excess is frequently found, 50 grams or more being often passed in the twenty-four hours. The increase is no doubt due, to a certain extent, to the large quantities of nitrogenous food consumed, but the experiments of Pettenkofer and Voit have shown that diabetics usually excrete more urea than normal individuals. Seegen concludes (*a*) that the urea excretion is increased in almost all cases of diabetes, but generally not markedly ; (*b*) there is no relation between the excretion of urea and sugar ; (*c*) the urea excretion is generally chiefly dependent upon the nitrogen of the food, and in only a few cases is it so abundant that it is necessary to consider that it is derived from the body proteins.

Hirschfeld has pointed out that in some cases of diabetes the resorption of nitrogenous material from the intestine, and with it the elimination of urea, may be very much below normal, and upon these grounds has advocated the recognition of a distinct form of diabetes characterised by a comparatively rapid course, the occurrence of colicky abdominal pains before, or at the onset, of the diabetic symptoms, a moderate degree of polyuria, and the existence of pancreatic lesions.

Ammonia.—The urine of a healthy person contains as a rule less than 1 gram of ammonia in the twenty-four hours, and normally only about 2 to 5 per cent. of the total nitrogen of the urine exists in the form of ammonia. The amount excreted depends, however, upon the diet to some extent. It is much diminished in vegetarians, in whom the ammonia nitrogen represents about 2 to 3 per cent. of the total urinary nitrogen, and is increased by a solely meat diet, when it may rise to 1·2 to 1·5 grams a day,

the ammonia nitrogen then representing about 5 per cent. of the total nitrogen. In diabetics, who are taking a large amount of meat, the ammonia nitrogen is increased above the average from that cause; but in some cases a quantity in excess of what can be accounted for by the nature of the diet is passed, while in others the output goes up without any increase in the nitrogenous food to 5 or 6 grams a day, representing 10 to 25 per cent. of the total nitrogen. In such cases there is a corresponding decrease in the excretion of urea. The reason for this has already been referred to, and will be more fully discussed when acidosis and diabetic coma are considered, since it is as a sign of these that an increase in the output of ammonia nitrogen is chiefly important.

Creatinin.—An increased excretion of creatinin in the urine, generally said to arise partly from the nitrogenous diet and partly from muscular wasting, is seen in many cases of persistent glycosuria, as much as 2 grams in the twenty-four hours being met with. Mendel and Rose state that carbohydrates, in contrast to other foodstuffs, are capable of preventing the excretion of creatinin, and are indispensable for creatin-creatinin metabolism. They found that experimental interference with carbohydrate metabolism, as in phloridzin diabetes, leads to the excretion of creatin, and increases the output of total creatinin (creatin plus creatinin). This increase is always accompanied by a rise in the total nitrogen excretion. They ascribe the parallelism between the total creatinin and total nitrogen outputs to true tissue, or endogenous, metabolism, while they came to the conclusion that the metabolism of exogenous, or reserve, protein is not accompanied by the production of creatin or creatinin.

Uric Acid.—It has been frequently stated that the urine in diabetes contains only a small amount of uric acid, but Naunyn and Reiss showed that this is an error due to incorrect estimation, and that the daily quantity is usually increased. This increase is, as a rule, dependent upon the exogenous uric acid derived from the meat diet, however, and Burian and Schur and others have found that in some instances the endogenous uric acid is diminished. Occasionally cases are met with in which there is a marked increase in the excretion of uric acid, amounting to as much as 3 grams in the twenty-four hours, associated with a diminution or disappearance of the sugar. To this condition the name " diabetes alternans " has been applied.

Xanthin Bases.—According to the observations of Bischofswerder, and of Jacoby, the xanthin bases (xanthin, guanin, &c.) are increased in most cases of diabetes.

Hippuric Acid and Benzoic Acid are both said to have been found in diabetic urines in demonstrable quantities.

Tyrosine is not met with in normal urines, but has been described as present in the urine of diabetic patients by Mies, Nicola, Adberhalden, and Möhr.

Indican.—According to Moraczewski, the amount of indican in the urine is increased in most diabetics, often very largely so. This is to be explained by the meat diet, and by the gastro-intestinal disturbance and hepatic insufficiency that are so common.

Glycocoll has been met with in the urines of two diabetics by Möhr.

Lactic Acid.—The quantity of lactic acid in normal urine is very small, under 0·02 grams per day, but after an abundant carbohydrate meal, and as a result of the administration of lactates by the mouth, the excretion may rise to five times the normal. In diabetes there may be an increase in the excretion of lactic acid in the urine, 0·05 to 0·06 grams being sometimes found. Both in health and disease it seems probable that the lactic acid that appears in the urine originates from the sugar of the blood through imperfect oxidation.

Oxalic Acid.—An abundant deposit of calcium oxalate crystals is often seen in both mild and severe cases of diabetes. Bose states they were detected in the sediment from the urine in 26·5 per cent. of his cases, and I have found them in over 20 per cent. of the cases that have come under my observation. Although an increased excretion of oxalic acid cannot be argued from the presence of calcium oxalate crystals in the urinary sediment, for other factors than the amount of acid determine their separation in crystalline form, it has been shown by quantitative estimations that the excretion of oxalic acid is frequently increased in diabetes. Thus Frerichs found 0·6 grams, as compared with the normal daily excretion of about 0·015 to 0·02 grams, in one case, and in this instance it was noticed that the output of oxalic acid and of uric acid ran curiously parallel. Barth and Autenrieth, Luzzato, and others have, on the other hand, reported cases in which the excretion was distinctly sub-normal. In some cases of diabetes a so-called " vicarious " elimination of calcium oxalate has been noticed, a diminution in the amount of sugar being associated with an increased deposit of oxalate crystals, and an increase in the sugar output being accompanied by a diminution or disappearance of the oxalates. This has been taken to indicate that glycosuria and oxaluria are two phases of the same condition, and that there is a relationship between carbohydrate metabolism and

oxalic acid. The experiments of Mayer tend to support this view, for he found oxalic acid in the liver and urine of rabbits after they had been injected with dextrose, or glucuronic acid. The frequent association of oxaluria with cirrhosis of the pancreas, which I pointed out some years ago, also suggests that the appearance of oxalates in the urine may be a result of the incomplete oxidation of carbohydrates. Since gastro-intestinal disturbances are very apt to be associated with oxaluria, probably in consequence of the defective digestion and subsequent imperfect oxidation of carbohydrates, the influence of such intercurrent conditions must be taken into account in explaining the oxaluria of diabetes and pancreatic disease, both of which are frequently accompanied by disturbances of digestion. The similar relations which the excretions of oxalic and uric acid bear to the intensity of the dextrosuria may possibly be accounted for by the fact that oxaluric acid, which is readily decomposed into oxalic acid, can be derived from uric acid.

Phosphoric Acid.—The quantity of phosphoric acid excreted in the urine is largely dependent upon the amount ingested, increasing with an animal, and decreasing with a vegetable, diet. It is also influenced by the amount of tissue destruction. The character of the individual phosphatic salts depends upon the alkalinity of the blood, and ultimately on the quantity of acid set free in the tissues, or absorbed during digestion. In many cases of diabetes the excretion of phosphates, chiefly the earthy phosphates, is considerably increased. Boecker in one case found that the earthy phosphates were about three times the normal, and Neubauer met with 0·711 grams of phosphate of lime, in twenty-four hours, in the urine of a child of six, an amount more than double the normal for an adult. Teissier drew attention to a curious relation that exists between the elimination of sugar and phosphates in some cases, the quantity of the latter rising and falling in inverse ratio to the former. Occasionally a condition known as " phosphates diabetes " is met with. In this, although sugar is absent from the urine, the patient presents various symptoms commonly associated with diabetes mellitus, and there is an increased excretion of phosphates, amounting to 7 or even 9 grams in the twenty-four hours.

Chlorides.—As the chlorides that are excreted in the urine are derived from the food, the animal diet is apparently the explanation of the increased output of chlorides that is generally seen.

Sulphates.—An increase in the excretion of sulphates in dia-

betes is, like an increase in the excretion of indican, to be accounted for by the abnormal putrefactive changes that go on in the intestine as a result of the highly nitrogenous diet and the associated gastro-intestinal disturbances ; for the etheral sulphates, to which the increase is largely due, are one of the means which the body adopts for harmlessly removing the toxic products of intestinal putrefaction.

Calcium.—A healthy adult excretes about 3 mg. of calcium per kilogram of body-weight daily, but in many diabetics, and more especially in the grave forms, this amount is very consider-ably exceeded, as Boecker, Dickinson, and others have shown. The excess has been attributed by Tenbaum to the nature of the diet, and, although this may be a partial explanation, there can be no doubt that a large part of the excess is derived from the osseous system in an attempt to neutralise the abnormal acids formed as a result of the perverted metabolism.

Iron.—The excretion of iron is increased in many cases of diabetes. Jolles and Winkler found in four cases that it oscillated between 83 and 136 mg. in the twenty-four hours. Neumann and Mayer state that the excretion of iron is proportional to that of sugar, and, although such a result would be of interest as bearing on the anæmia of diabetes, it has not as yet been confirmed.

Albumen.—Some authors consider that albuminuria is frequently met with in diabetes, while others are of the contrary opinion. This difference appears to depend partly upon the interpretation of the term that has been adopted, and partly upon the class of case from which the statistics have been drawn. If only a decided reaction for albumen, such as is given by 1 gram or more per litre, is taken into account, it is found that only some 8 or 10 per cent. of cases suffer from albuminuria, according to the extensive experi-ence of Külz and Lépine. If, however, a slight opalescence is also accepted as evidence of albuminuria, some 66 per cent. of cases must be included, as in Schmitz's statistics. For clinical purposes it is therefore advisable to divide the cases into two groups : (1) those in which only a small quantity of albumen is present, and there are no other indications of kidney mischief ; (2) those in which there is a characteristic reaction for albumen, and in addition other signs of nephritis.

The former are by far the most common. They include those cases in which, according to Naunyn, the albuminuria arises from the effect of the glycosuria on the kidneys, and also those in which a trace of albumen comes from a small amount of purulent material, mixed with the urine, that is derived from the discharge exuded by the external genitals consequent on the irritation of the saccharine

urine. Slight albuminuria is also often observed when phthisis is a complication of diabetes, and in elderly diabetics a small quantity of albumen is not infrequently met with in the urine, accompanied by an excess of uric acid. It has been pointed out by Lépine that albuminuria is particularly frequent in traumatic diabetes, and he suggests that the appearance of albumen in such cases is the result of nervous influences.

In members of the second group there are all the indications of parenchymatous, or interstitial, nephritis, the disease of the kidney being in some cases, however, probably antecedent to the glycosuria. A large quantity of albumen along with casts in the urine, œdeme, headache, retinitis, and cardio-vascular changes points to a parenchymatous inflammation of the kidneys. In such cases it is often found that as the albumen increases in amount, the sugar diminishes, until only the former remains. In other cases the symptoms of granular kidney are present, and here again the sugar may disappear as the kidney lesion advances. In either type of case the disappearance of the sugar is a grave prognostic sign. According to Williamson, albuminaria is more common in private practice than in hospital cases, where, as a rule, the appearance of albumen in the urine is a late symptom. A very common sign of approaching diabetic coma is the appearance of albumen. generally accompanied by many granular and hyaline casts in the urine. According to Maguire, albuminuria is always present in diabetic coma.

The Acetone Bodies.—With the exception of sugar itself, the acetone bodies are the most important substances met with in the urine in persistent dextrosuria, for their presence signifies that the case has reached the second stage in its downward progress.

Acetone is the only one of the three that is met with in normal urine, but never in quantities that can be recognised by the ordinary clinical tests. It is the first to appear in appreciable amounts when acidosis is developing. Some authors contend that acetone is never preformed in the urine, but is always derived from acetoacetic acid, and although this view is not generally accepted, it cannot be denied that a part of the acetone obtained by distillation processes from diabetic urines comes from the contained aceto-acetic acid. According to Embden and Schliep, at most a quarter. often a sixth. and very frequently a tenth, only, of the acetone is preformed.

Normal urine contains very little acetone, 0·01 to 0·03 grams in the twenty-four hours, but in chronic glycosuria it may rise to 0·5 grams, and in severe cases of diabetes may reach 1·0 to 4·5 grams, or, with a purely protein diet, even 5 grams in the twenty-four

hours. In the latter case a great part of the acetone is undoubtedly derived from aceto-acetic acid. A marked increase in the excretion may follow slight fever. Acetone also leaves the body in the expired air, as much as 150 mg. an hour being sometimes got rid of in this way. The acetone in the breath is usually considered to be the cause of its characteristic sweet smell in severe cases of diabetes, but this is denied by Folin.

Aceto-acetic acid appears in the urine in relatively small quantities, rarely exceeding 10 per cent. of the total organic acids ; but, owing to the ease with which it is converted into acetone, it is not easy to determine the exact proportion in which the two occur. They are therefore frequently estimated together.

Beta-oxybutyric acid is met with in very variable quantities, the amount depending on the extent of the secondary disturbances of metabolism that are present. In mild cases of persistent glycosuria it is usually absent, but in severe cases of diabetes large quantities are found as a rule. Preceding the onset of diabetic coma 15 to 20 grams a day may be met with, and Külz has reported that in three cases a daily output of 67, 100, and 226 grams respectively was observed. When coma has developed it is frequently found that the elimination does not keep pace with the formation of the acid, so that considerable quantities are retained within the body, and the quantity in the urine drops. Every urine that contains oxybutyric acid also contains acetone and aceto-acetic acid, but the converse is not true. In a few exceptional cases a certain parallelism is seen between the excretion of beta-oxybutyric acid on the one hand, and aceto-acetic acid on the other for a few days, but an inverse relationship is more commonly found, as might be expected. As a rule, when once oxybutyric acid has appeared in the urine it increases at a much more rapid rate than the aceto-acetic acid and acetone, so that 30 to 80 grams may be present, while the total amount of the other two rarely exceeds 7 to 8 grams. The parenchymatous degeneration of the heart, kidneys, and sometimes of the liver, seen in fatal cases of diabetes, is attributed by Busse to the action of beta-oxybutyric acid.

The Blood in Diabetes.—Many observers have found that in cases of diabetes, of apparently equal severity, the number of erythrocytes, and the hæmoglobin content of the blood, may be increased in one, and diminished in another, or may vary considerably from time to time in the same patient. Most recent investigations have shown that, while there may be considerable individual variation in the percentage of hæmoglobin, it does not

differ greatly from the normal when a series of cases is considered, and the same may be said of the red blood cells, for, although they are often increased, this is not a constant condition, and in some instances they are found to be diminished. Leichenstern, finding an excess of hæmoglobin in an advanced case and a diminution in an early case, was led to consider that the determining factor is the concentration of the blood, which in its turn depends upon the diuresis. This explanation has been generally accepted, and it is concluded that any increase in the number of erythrocytes is also to be referred to this cause. James, who carefully investigated the blood in thirteen cases of diabetes, found that in five of them the red corpuscles were increased to six millions, or more, per cubic millime re, in five they were normal, in two they were four millions per millimetre, and in one three millions per millimetre, but con- cluded that the specific gravity of the blood was not distinctly raised in those with an excess, as it should have been were the concentra- tion of the blood the only cause of the polycythæmia. Ewing criti- cises this conclusion, and considers that the specific gravities cited are distinctly above normal if allowance is made for the percentage of hæmoglobin found, and states that James' results indicate a relative anhydremia with marked reduction of hæmoglobin, and but slight loss of red cells. Although it may be allowed that the marked changes seen in the blood of many diabetics may be referred to the uncertain balance between the amount of water absorbed and that excreted in the urine, other factors undoubtedly enter into the problem in the later stages, for the general failure of nutri- tion that occurs must affect the blood. Even in extreme cases, however, the anæmia that results is frequently masked by the concentration of the blood consequent on excessive diuresis.

As a rule there is no definite change in the number, or relative proportion, of the leucocytes in diabetes, although, according to v. Limbeck, digestion leucocytosis is often very well marked. Gabritschewsky has drawn attention to the existence of an excess of " glycogen " in the leucocytes, and plasma, in severe cases. This is demonstrated by mounting cover-glass preparations in a solution of iodine (1 part), and potassium iodide (3 parts), in water (100 parts), containing an excess of gum-arabic. The significance of the brownish extra cellular granules, which are stated to be two or three times as numerous as in normal blood, has been questioned, however, and it has been pointed out that myelin, lecithin, &c., also stain brown with iodine. Their significance is therefore not certain. According to Locke, the leucocytes only contain the granules in pathological conditions, and they are particularly

abundant in diabetes when coma, or gangrene, exists ; but as they are also found in other diseases, sometimes in large numbers, and more especially when there is septic absorption going on, they are of little diagnostic value. The granules are found chiefly in the polymorphonuclear neutrophiles, but also to some extent in the large and small mononuclear cells. According to some authors they are seen in the eosinophile cells only in diabetes, but Habershon believes that the eosinophile granules themselves are related to, or are identical with, glycogen, and he states that normally from 1 to 16 per cent. of all leucocytes contain glycogen granules. Futterer claims to have demonstrated thrombi composed of glycogen in the brain and medulla in diabetes.

To the naked eye the blood in diabetes usually presents no striking variation from the normal, but in rare instances it may have a pink colour, or an appearance like *chocolat au lait*. In such cases a milky, or cream-like, serum separates out on standing, and analysis may reveal as much as 19 per cent., 20 per cent., or even more of fat, instead of the normal of about 0·1 to 0·2 per cent. Chemical analysis shows that as a rule the fat consists largely of olein, with a small amount of free fatty acid, but in some cases a considerable proportion of cholesterin has also been found, a quarter of the total in a case quoted by Javal. Microscopically the fat is seen to be in the form of fine granules, which stain feebly with osmic acid ; but as all that stains with osmic acid is not fat, a control specimen, that has been thoroughly extracted with alcohol and ether previous to staining, should be compared with the original sample. Even in cases where the blood appears normal to the naked eye an excess of fat may be often demonstrated by the use of the microscope, or the hæmatokrit. The occurrence of lipæmia in alcoholism, pneumonia, anæmia, and phosphorus poisoning, in all of which there is defective oxidation, as well as in diabetes, suggests that an impairment of the power of the organism to oxidise fats is the cause of the condition ; but, as we are not acquainted with the essential steps that take place in the oxidation of fat, it is not possible to suggest how the failure of fat destruction is brought about. The origin of the fat in lipæmia is likewise uncertain. Ebstein considers that it arises partly from the food, and partly from the fatty degeneration of the cells of the blood, the vessel walls, and the viscera. The presence of a considerable excess of cholesterin in some instances tends to favour the view that some portion at least has the latter origin. Neisser and Derlin conclude that it is merely fat from the food, coming from the chyle that accumulates in the blood. Fischer believes that

it is largely derived from the fat stores of the body, but that, owing to a loss of lypolytic power on the part of the blood, it cannot be rendered diffusible and so enter the tissues, where it is normally consumed. It has been stated that diabetic coma and death in diabetes may be caused by fat embolism of the cerebral vessels. While this is possible, it cannot be a common occurrence, for a marked degree of lipæmia is rare, and even then the fat droplets are too small to cause occlusion of the vessels unless they combine to form large droplets. Fischer doubts whether this can ever occur, and supports his contention by experiments and clinical records.

The alkalinity of the blood in normal individuals varies between 300 and 400 mg. of sodium hydrate per 100 grams of blood. In diabetics with little general disturbance it is found to be slightly reduced, but in cases with marked general disturbance of metabolism it is lower than in any other known condition, falling to 40 mg. or so of sodium hydrate per 100 mg. of blood (v. Noorden). The reaction of the blood is, however, never acid to litmus. Brandenburg has pointed out that a distinction must be drawn between the total alkalescence of the blood, referable to the non-diffusible combinations of alkalies with albumen, shown by direct titration, and the " alkaline tension," due to the diffusible alkalies, indicated by the proportion of carbon dioxide obtainable by dissociation of the carbonates. This distinction is particularly important in diabetes, for it is chiefly in diabetic coma that a marked reduction in the alkali tension is met with. Thus in one case Minkowski found as little as 3·3 volumes of carbon dioxide per cent., as compared with the normal 33·37 to 45·3 per cent. This relative acidæmia is referable to the presence of various acid products of fat and protein metabolism, including beta-oxybutyric and other fatty acids.

The chief chemical alteration in the blood in diabetes is the increase in the amount of sugar it contains. While the sugar content of normal blood varies from 0·05 to 0·15 per cent., Pavy and Seegen have found as much as 0·6 per cent. in cases of severe diabetes, and Hoppe-Seyler reported 0·9 per cent. in one instance. Frerichs found the sugar in the blood in diabetes to vary between 0·38 and 0·44 per cent. when the urine contained from 5·5 to 8·4 per cent. Naunyn met with 0·7 per cent. of sugar in the blood of a fatal case of diabetes in which the urine contained 4 per cent. Henriques and Kolish have claimed that an excess of preformed sugar exists in the blood only in alimentary glycosuria, and that in diabetes it is but slightly above the normal. They consider

that in the latter condition most of the sugar exists in combination, the blood containing a marked excess of jecorin combined with albumen, which is split up in the kidneys during excretion into dextrose and lecithin.

Several tests have been devised with the object of differentiating diabetic from non-diabetic blood.

Williamson's Test.—Williamson showed that diabetic blood, like diabetic urine, decolorises solutions of methylene blue, while normal blood does not, and he suggests that this test may be useful in cases where a specimen of urine is not available.

The test is performed as follows :—20 cm. (2 drops) of blood are mixed with 40 cm. of water, and added to 1 c.c. of methylene blue (1 : 6000), and 40 cm. of liquor potassæ (sp. gr. 1·058), in a small narrow test-tube, and well mixed by shaking. A control specimen of normal blood is then prepared in the same way. Both test-tubes are suspended in a beaker of water, the contents of which are brought to the boiling-point, and the boiling continued for four minutes. The specimen containing the diabetic blood is then seen to have changed from a fairly deep blue to a dirty, pale yellow, while the specimen in which the normal blood has been added remains blue, or occasionally assumes a bluish-green, or violet, tint. It is important that the tubes should not be shaken while they are being heated, or after the experiment is completed, as the colour of the diabetic specimen may be restored through the action of the atmospheric oxygen. Williamson obtained a positive result with all of fifty specimens from twenty cases of diabetes, but failed to obtain the reaction in over 100 cases of other diseases.

Bremer's Test.—Another test, introduced by Bremer, has also been supposed to depend upon the hyperglycæmia, but it is more probably due to the presence of abnormal acids, as Schneider and others have shown that the reaction runs parallel with the quantity of these present in the blood.

This test is carried out as follows. A smear preparation of the blood is fixed, by heating it at 60° C. in equal parts of alcohol and ether for four minutes. It is then stained for four minutes in a freshly prepared solution containing 0·025 to 0·05 grams of a powder (made by mixing twenty-four parts of the dried and washed precipitate formed when saturated aqueous solutions of eosin and methylene blue are mixed, in about equal proportions, with six parts of methylene blue and one of eosin) in 10 c.c. of 33 per cent. alcohol. After washing the stained preparation in water, diabetic blood should have a greenish tint, while normal blood is reddish-violet. On microscopical examination the erythrocytes in diabetes should appear greenish, normal erythrocytes red.

Various modifications of this test have been introduced from time

N

to time. Bremer found that 1 per cent. solutions of Congo red, or of methylene blue, acting for one and a half to two minutes, stain diabetic blood, that has been fixed by heating to 125° C. for six to ten minutes, very slightly, but that a 1 per cent. solution of Biebrich scarlet stains it very intensely. Directly opposite effects are obtained with normal blood. Rather thick smears should be employed, and the preparations must not be heated to over 140° C. The colours should be compared with the naked eye. Bremer's results have been confirmed by other observers, but similar reactions have been obtained with the blood in leukæmia, Hodgkin's disease, exophthalmic goitre, and multiple neuritis, and a partial reaction in cachectic conditions. Yet in most conditions other than diabetes the reaction has been found to be inconstant, and to occur only in a small proportion of the cases. It is to be noted that a slight variation in the technique appears to vitiate the results of both Williamson's and Bremer's tests.

Clinical Symptoms of Persistent Dextrosuria.—In many cases of persistent dextrosuria the presence of sugar in the urine is the most prominent, and, in some, the only sign of the metabolic disturbance that exists, so that the condition is only discovered accidentally in the course of a routine examination for life insurance, or for some other purpose. This is particularly the case when the patient is middle-aged or stout. Inquiry may show that there has been some loss of weight, but often there is no sign of it, and with the exception, perhaps, of a feeling of lassitude, or a general loss of mental and physical tone, no special symptoms can be discovered. In others the occurrence of one or other of the numerous complications to which diabetics are liable, may draw attention to the presence of sugar in the urine.

With severe cases the clinical picture is very different. It is obvious when the patient is first seen that he is suffering from some serious wasting disease ; his form is emaciated, his face is pale and sunken, the naso-labial folds are deep and well-defined, and are frequently prolonged round the angles of the mouth. There is sometimes a dull red flush on the cheeks, and the tip of the nose and lips are slightly cyanosed. The expression is listless or anxious, and the patient looks older than his years. The skin and hair are often dry and harsh. The tongue may be moist and covered with a thin yellowish white fur, or may be unnaturally clean, red, glazed, and beefy-looking. Sometimes it is fissured and cracked. The mouth is dry, and thirst is often a prominent symptom. The gums are frequently red and inflamed, and the teeth loose. A voracious appetite, which is only temporarily assuaged by food, may be present, yet, in spite of the large quantities of food and drink consumed, indigestion is not complained

of as often as might be expected. On being weighed the patient is found to be considerably below the standard for an individual of his height. Physical examination of the abdomen usually shows nothing abnormal, although rarely a fullness or swelling may be detected in the region of the pancreas.. No physical signs of disease are found in the chest, unless as the result of tuberculosis and other complications, which will be mentioned shortly. The temperature is generally normal, or slightly subnormal.

In many instances *the family and past history* of the patient throw little or no light on the condition, but in some 18 to 20 per cent. it will be found that one or more blood relations have suffered from glycosuria, or died of diabetes. Williamson states that the relatives most frequently affected in order of frequency are—brother. father, mother, and sister. In five out of 250 of his cases (2 per cent.) the husband or wife of the diabetic also suffered from glycosuria, but of course such instances cannot be included in a table of heredity. Williamson records some striking examples of this family tendency. In one family, consisting of two sons and two daughters, both the sons and one daughter became diabetic. Each had lived in a different town, and several years elapsed between the onset of the glycosuria in the three cases. I have had under my care a patient who informed me that his father, grandfather, and two uncles all passed sugar in their urine, but all of them died of some other disease than diabetes.

Loeb considers that cases in which heredity can be traced are distinct as regards etiology, symptoms, course, complications, and prognosis—that is to say, there is an *hereditary variety of diabetes*, which can be differentiated by the following characters :—
1. *Etiology.*—(*a*) Females in hereditary cases are affected as frequently as, or even more frequently, than males. In diabetes generally males are affected about thrice as frequently as females. In Frankfort, where hereditary diabetes is exceedingly common, the diabetes death-rate is about the same for males and females ; in one year more females than males succumbed. In a family tree, constructed by von Noorden, there were eight cases of diabetes in females and only five in males ; and in a diabetic family Loeb found eight cases in females and four in males. The statistics of Weidenbaum, based on one thousand cases of diabetes, point in the same direction. (*b*) As regards race, the hereditary form of diabetes is especially frequent in Jews. Wallach showed that in a period of twelve years the proportion of deaths from diabetes was six times greater among Jews than Gentiles, the greater frequency of the hereditary form among Jews probably accounting

for the whole of the excess.   (c) The hereditary form of diabetes seldom occurs in youth, and usually appears between fifty and sixty.   Loeb has seen it only twice in young people—once in a woman aged twenty-three, and once in a boy of ten, whose aunt had died of diabetes at thirty.   Von Noorden knew of a family in which a slight case of diabetes occurred ; in the second generation three female members were attacked in middle age by a rapidly fatal form of diabetes, and in the third generation two children fell victims to the disease.   (d) As regards constitution, the hereditary form almost always attacks well-nourished previously healthy subjects.   Frequently, and especially in women, there is a tendency to obesity, though the diabetes of obese subjects is not necessarily hereditary.   Many of these patients are " nervous " or suffer from paralysis agitans, hysteria, or mental disease.

2. *Course.*—In hereditary diabetes the onset is often insidious. Sugar may recur at varying intervals in the urine, usually in small, though sometimes in large, amounts.   The general health at this early stage is not affected.   The duration of the disease cannot readily be estimated, as it often exists for years before the onset of general symptoms.   Usually it runs a chronic and benign course, so that if the patients are docile and escape intercurrent diseases, they may attain a good age.   Acetone bodies are seldom excreted in excess until shortly before death.

3. *Symptoms.*—The symptoms are those of a mild form of diabetes, and are readily controlled by diet.   They recur after errors of diet, or excitement, and may eventually persist.   Arteriosclerosis, as evidenced by thickening of the radial artery, high blood pressure, and hypertrophy of the left ventricle, or, in more advanced cases, by asthmatic and anginal attacks, cerebral hæmorrhage, and albuminuria, usually occurs early in hereditary diabetes. Pulmonary tuberculosis is practically unknown in the hereditary form.

4. *Complications.*—The dangers which threaten patients with the hereditary form of diabetes are chiefly those of intercurrent diseases (influenza, pneumonia, erysipelas, arterio-sclerosis, and its results—gangrene, thrombosis, and cardiac failure).   In only two of sixteen fatal cases was death directly attributable to diabetes.

In some cases of persistent glycosuria there is a family history of gout, diabetes insipidus, phthisis, exophthalmic goitre, epilepsy, or various neuroses.

The glycosuria will in some instances be found to have made its appearance after a period of acute or prolonged nervous strain,

such as severe anxiety, grief, or business worry. Occasionally an attack of some infectious disease, such as typhoid fever, or syphilis, may be considered to be the starting-point, and with regard to these it is important to remember that glycosuria may not appear for some years after the attack, for in such cases the pancreas is probably the organ at fault, and the degree of inflammatory change necessary to give rise to glycosuria is only slowly produced. In one case that has come under my care sugar was found in the urine, and typhoid bacilli were isolated from the fæces ten years after an attack of typhoid fever.

On September 8, 1908, I was consulted by an American gentleman, who came to me with a letter of introduction from Dr. J. B. Murphy of Chicago. In his letter Dr. Murphy stated that the patient was suffering from diabetes, and had been sent to me with the object of determining the condition of his pancreas and clearing up the etiology of the disease. He also informed me that there was a distinct history of cholecystitic infection.

The patient was a well-built, healthy looking man, fifty-one years of age. He had had no serious illness, except an attack of typhoid fever in 1898. There was no history of syphilis, or gastro-intestinal disturbances, nor had he had any symptoms pointing to the presence of gall-stones. Although he smoked a good deal, he took little or no alcohol. He had never suffered from thirst or polyphagia. The quantity of urine excreted had not increased. So far as he knew he had not lost flesh, nor had he noticed any diminution of strength. Three per cent. of sugar had been found in his urine in the course of a routine examination at an American health-resort in January 1908, but on an anti-diabetic diet this had been reduced to about half.

Physical examination revealed nothing abnormal. The patient's tongue was not red or glazed, and his gums and mouth appeared normal. His skin was moist. His heart was not enlarged, the heart sounds were natural, and his arteries were not thickened. His abdomen and body generally were well covered with fat. No abdominal tumour or swelling could be discovered on palpation. The liver dullness was normal, and the stomach did not appear to be dilated. Analysis of a specimen of the mixed night and morning urine gave the following results :—

Reaction, acid ; *Specific gravity*, 1023 ; *Albumin* about 1 : 5000 ; *Sugar*, Fehling's solution—reduced at once, phenylhydrazin—crowds of typical phenylglucosazone crystals, insoluble in 33 per cent. sulphuric acid in five minutes : quantitatively, copper reduction (Bang's method), 2·3 per cent., polariscope, +2·2 per cent. ; fermentation (Lohenstein's saccharimeter), 1·9 per cent. ; *Acetone*, nil ; *Aceto acetic acid*, nil ; *Indican*, a fairly well-marked reaction ; *Bile*, nil ; *Urobilin*, a pathological excess ; *Blood*, nil ; *Urea*, 2·42 per cent. ; *Chlorides*, 0·8 per cent. ; *Phosphates*, 0·13 per cent. ; *Preformed to*

Conj. sulphates, 8 : 1. " *Critical solution point* " (phenol), raised 10° C. *Microscopically*, many small calcium oxalate crystals, a few squameous and transitional epithelial cells, a few leucocytes, no casts. " *Pancreatic* " *reaction*, many typical fine crystals, soluble in 33 per cent. sulphuric acid in five to ten seconds ; melting-point, after recrystallisation, 160° C.

The urine, therefore, contained a fair amount of a dextro-rotatory fermentable sugar, with traces of a non-fermentable variety. The results of the " pancreatic " reaction suggested that the glycosuria was probably associated with disease of the pancreas, and the presence of many small calcium oxalate crystals in the centrifugalised deposit tended to confirm this conclusion. The pathological excess of urobilin pointed to there being a catarrhal condition of the biliary passages, which probably extended to the pancreatic ducts. The abnormal reaction for indican, and the disturbed relation of the pre-formed to the conjugated sulphates, suggested that both the pancreatic disease and cholangitis were connected with a catarrhal condition of the upper part of the intestinal tract. The absence of any trace of acetone, or aceto-acetic acid, showed that there were not the profound tissue changes met with in severe diabetes, and rendered it probable that the pancreatic disease was of a slowly advancing type, such as, in my experience, is the common result of cirrhoses of the pancreas secondary to infection of the ducts. Although the specimen contained traces of albumin, the fairly normal critical solution point, the normal percentages of urea and inorganic salts, and the absence of casts or other evidence of renal mischief, rendered it unlikely that there was any serious disease of the kidneys, while the presence of leucocytes and transitional epthelium suggested that it was not improbably of bladder origin.

The bowels were stated to be opened regularly every day, and there was neither diarrhœa nor constipation. A sample of the fæces submitted for examination gave the following results :—

Appearance, dark brown, formed, solid mass ; *Reaction*, amphoteric ; *Stercobilin*, a well-marked reaction ; *Occult blood*, nil. *Microscopically*, a little vegetable tissue, many partly digested muscle fibres, some fatty acid and soap crystals, no fat globules : *Quantitative analysis* showed :—

Organic matter	88·7	per cent. of the dry weight			
Total fat	16·6	,,	,,	,,	,,
" Unsaponified " fat [1]	8·6	,,	,,	,,	,,
Saponified fat	8·0	,,	,,	,,	,,
Organic matter not fat	70·1	,,	,,	,,	,,
Inorganic ash	13·8	,,	,,	,,	,,

The only striking variation from the normal was the large amount of partly digested muscle fibre found microscopically, but this was probably to be explained by the highly nitrogenous diet which the

---

[1] *i.e.* neutral fats and free fatty acids estimated together. See *Brit. Med. Journ.*, Oct. 28, 1905, p. 1102.

patient was taking. There was no marked excess of unabsorbed fat, nor was the relation between the saponified and " unsaponified " fats disturbed in the way that one might expect in serious disease of the pancreas. This, however, I have found in a considerable number of cases of glycosuria of undoubted pancreatic origin in which I have had an opportunity of examining the stools, and I have come to the con- clusion that any marked interference with fat digestion is of serious prognostic significance, and indicates that the patient is in a late stage of the disease. The absence of any trace of occult blood was against there being any malignant growth in the course of the gastro- intestinal tract, to which the pancreatic disease might be secondary.

Bearing in mind the history of typhoid fever, and the well-re- cognised tendency of the typhoid bacillus to linger in the gall-bladder and bile-ducts, in some cases, long after the patient has quite recovered from the disease, it seemed to me possible that I had to do with a case of typhoidal pancreatitis going on to glycosuria, and that a bacterio- logical analysis of the fæces might throw further light on the condition. A portion of the fæcal material was taken with a sterile knife from the centre of the mass sent for examination, emulsified in sterile normal saline solution, plated on Drigalski and Conradi's medium, and in- cubated at 37° C. On being examined twenty-four hours later one of the six plates that had been inoculated showed two small, blue, finely granular colonies, with raised centres and filmy edges, suggesting bacillus typhosus. Sub-cultures were made from these on to agar- agar slopes, and incubated at 37° C. for twenty-four hours ; they then showed a thin bluish white, transparent film, that had not spread far from the needle track. Hanging-drop preparations showed actively motile rod-shaped bacilli. The bacilli did not stain by Gram's method, and cover-glass preparations stained by Van Ermengen's method showed that they were richly flagellate. Glucose-gelatine shake cultures showed no gas formation in forty-eight hours. Lactose litmus broth was not rendered acid, and showed no gas formation. Litmus-milk cultures were not coagulated, nor was there any acid fermentation in forty-eight hours. Neutral red broth became turbid, but there was no film formation or colour change. Peptone salt cultures incubated at 37° C. for forty-eight hours gave no indol re- action. An emulsion of the bacilli in normal saline solution, prepared from a twenty-four hours agar culture, gave a similar reaction with a typhoid serum to a laboratory culture of bacillus typhosus. The fæces, therefore, contained a small number of bacilli having the appear- ance and characters of bacillus typhosus ; a result which tended to confirm my surmise as to the probable origin of the disease.

In a few cases the glycosuria may be traced to changes set up in the pancreas by gall-stones. One of the most common exciting causes in my experience is chronic gastro-enteritis. This has often not been serious, but on going carefully into the history of cases of glycosuria it is not infrequently found that there have

been symptoms of " intestinal indigestion " extending over many years, and that the patient has been abnormally fond of sweets. Funck has also remarked that gastro-intestinal disturbances are unexpectedly prominent in the histories of diabetics when they are carefully gone into, and suggests that gastritis and chronic enteritis are a primary factor in the production of chronic glycosuria in many cases, or are at least responsible for exacerbations much more frequently than is generally supposed.

Owing to the special nature of my work for a considerable number of years I have seen an unusually large proportion of cases in which glycosuria has been associated with disease of the pancreas and gall-stones. Taking a consecutive series of two hundred cases I find that biliary calculi were present in just under 12 per cent., and it is noteworthy that in nearly half of these (47 per cent.) there was no jaundice, and that there was more sugar than in the jaundiced cases, suggesting that the absence of this striking symptom had deferred the diagnosis and led to more serious pancreatic mischief. Eight of my cases (4 per cent.) had been operated on for gall-stones at periods varying from two to six years prior to the onset of the glycosuria. In one instance the presence of sugar in the urine was associated with a growth of the ampulla of Vater, in one with a growth of the duodenum invading the pancreas, and in twelve (6 per cent.) with primary malignant disease of the gland. In two cases there was a cyst of the pancreas, and in two pancreatic calculi were found, in one at operation and in the other post-mortem. In one case a transient glycosuria was associated with parotitis and symptoms of pancreatitis. I found interacinar pancreatitis in three and interlobular pancreatitis in two cases not associated with gall-stones. One of the latter had been diagnosed as malignant disease during life, but post-mortem no secondary growths could be found, and histologically there was no evidence of cancer. Interlobular pancreatitis was also found in three cases of gall-stones with glycosuria that I investigated. In one of my cases there was a history of an accident in which the upper part of the abdomen was crushed eight months before the onset of the glycosuria. Arterio-sclerosis was present in 6 per cent. of the cases, a history of gout was obtained in 4 per cent. Two had suffered from syphilis, one had exophthalmic goitre, one was a member of a family that suffered from hæmophilia and was a " bleeder " himself, in one the glycosuria had come on during pregnancy, four, including the one already mentioned, gave a history of typhoid fever, and two had had repeated attacks of influenza. In six cases (3 per cent.) glycosuria appeared

to be a family disease, and had affected one or more members beside the patient. A history of chronic indigestion was given by 10 per cent. of the cases, and an analysis of the urine and fæces tended to show that there was a chronic catarrh of the intestine. In four cases there was a definite history of duodenal ulcer.

Complications.—Persistent glycosuria is not of itself a serious condition, and many patients whose urine contains sugar live comfortable lives for many years ; it is the complications and secondary effects of the metabolic disturbance to which the glycosuria is due that constitute the danger. All the complications met with in diabetes do not threaten the existence of the patient ; some are merely annoying or painful, but the appearance of others is of the very gravest import, and unless they are speedily recognised, and treated, a fatal termination is likely to quickly supervene.

The secondary effects of persistent glycosuria may be conveniently considered under the various systems.

The Skin.—The skin in mild and early cases appears and feels normal, but in severe cases of diabetes it is usually harsh and dry, in striking contrast to the velvety feel sometimes met with in diabetes insipidus. Occasionally, however, it may be moist, and perspiration may be excessive, in cases that are otherwise typical. Some observers state that they have detected sugar in the sweat, but others have failed to do so. In one case sugar is said to have been found in the tears. *Pruritis*, which may be general, but is more frequently confined to the external genitals, and the skin in their neighbourhood, is sometimes troublesome, and occasionally is one of the first symptoms. Local pruritus about the genital organs is most common in women and may draw attention to the existence of the glycosuria, especially in stout females. The irritation of the skin is generally accompanied by congestion and redness, which may lead to balanitis in men, and vulvitis in women, together with *eczema* of the neighbouring skin. The majority of cases of eczema of the vulva occurring in women about the climacteric are due to glycosuria. Sometimes eczema and erythema also occur in other situations. *Psoriasis*, *urticaria*, and more or less localised patches of *œdema* are also met with in some cases. *Xanthoma* is a very rare complication, which, when present, diminishes as the sugar in the urine is reduced, and reappears with a return of the glycosuria. *Boils* are among the most common skin lesions in chronic glycosuria. They often occur at an early stage, and may be the first obvious symptom of the condition. It is therefore important that the urine of everyone suffering from

furunculosis should be repeatedly and carefully examined for
sugar. The boils may be met with in any situation, but are most
common on the neck, the shoulders, and the buttocks. According
to Marechal one-third, and v. Noorden one-fourth of all persons
having boils suffer from glycosuria. In advanced cases of diabetes
they are rare. *Carbuncles*, which like boils may be amongst the
earliest symptoms, also occur later in the disease, when they have
a tendency to spread and become gangrenous, or to give rise to
cellulitis, so that they are sometimes the cause of death. They
are most commonly seen on the neck, but they also occur on the
face, or other parts. Carbuncles are a less frequent complication
than boils. *Gangrene* is sometimes met with, and occasionally
may be the first symptom of diabetes; it may come on spon-
taneously, or as the result of slight injury, or be secondary to
wounds, boils, carbuncles, &c. The lower limbs are most fre-
quently affected, the gangrene commencing in the toes. In a
large proportion of cases of gangrene of the leg the lumen of the
vessels is reduced by atheroma and the blood supply is conse-
quently diminished, hence the *intermittent claudication*, or limping,
first noticed by Charcot. The gangrene may be moist or dry. With
the former the constitutional symptoms, such as loss of appetite,
drowsiness, and delirium are more marked than with the latter.
Perforating ulcers, resembling those met with in locomotor ataxia,
are occasionally seen in diabetes, and are probably of nervous
origin. They are chiefly seen on the soles of the feet, and are
especially common about the big toe. The starting-point is fre-
quently a wound caused by cutting a corn, &c. All *wounds heal
badly* in diabetic patients, so that operative interference is avoided
as far as possible by most surgeons.

Digestive System.—In severe cases of diabetes the *breath* has a
peculiar sweet smell, like that of decomposing apples. It is
generally referred to the presence of acetone, although this is
doubted by some authorities (Folin). The *mouth is often dry*,
and the saliva is, as a rule, scanty. Rarely ptyalism, such as is
occasionally met with in association with disease of the pancreas,
has been noted. The saliva is usually said to be free from sugar,
although this is denied by Redier, is acid in reaction, and some-
times does not give the sulphocyanide reaction. The *gums* are
often spongy, tender, and retracted. The *teeth* are frequently
carious, without giving rise to much pain. Alveolar periostitis
occasionally occurs, and the teeth may become loose and drop out
as the disease progresses. In advanced cases of diabetes aphthous
stomatitis is sometimes present. The *pharynx* may be intensely

congested, especially about the base of the tongue, and this congestion may spread to the larynx. The *tonsils* may be the seat of abscess or gangrene. The *appetite* is often, but by no means always, increased. This increase is met with chiefly in the severe forms, or in mild cases when carbohydrate food is being taken in large quantities. The excess of food is liable to cause dilatation of the stomach and may set up *gastritis*, which in its turn may result in atrophy and absence of gastric juice. Grübe and others have observed *abdominal crises*, resembling those seen in locomotor ataxia. The patient is suddenly seized with violent abdominal pain, especially at the pit of the stomach, the abdomen becomes distended, eructations occur, nausea and the vomiting of acid material follow, and this is sometimes succeeded by diarrhœa and cramp in the legs. Such a condition is not infrequently the precursor of diabetic coma. Diabetic patients often suffer from *constipation*, especially when they are on a highly nitrogenous diet, and when diabetic coma is imminent. Occasionally symptoms so closely resembling those of acute intestinal obstruction that immediate operation appeared necessary have been met with. Downes and O'Brien have reported two such cases, and it was only when the urine was examined and found to contain a large amount of sugar, beside giving well-marked reactions for acetone bodies, that the true nature of the condition was recognised. Similar cases have also been described by Tirard and Davies. *Diarrhœa* may sometimes result from intercurrent tubercular enteritis, but is more often dependent upon mal-assimilation of the fatty and nitrogenous foods prescribed. In a few cases typical *fatty stools* are seen, but steatorrhœa is rare, even when the glycosuria is associated with pancreatic disease. In a consecutive series of a hundred cases of diabetes, on ordinary diet, in which I have made an analysis of the fæces, an abnormal proportion of unabsorbed fat was only found in sixteen, but in forty-eight cases an excess of " unsaponified " over saponified fat was met with. Undigested muscle fibres were discovered microscopically in five cases, and a deficiency of pancreatic juice was shown by the casein digestion test, in fifty-two. In thirty-four of these it was slight, and in eighteen well marked. On rare occasions an *enlarged pancreas* can be made out on abdominal examination, especially in thin subjects and under an anæsthetic. This may be due to inflammatory changes in, and around, the gland, when there is likely to be tenderness in the epigastrium, and a tender spot, just above and to the right of the umbilicus, with possibly pain in the back under the right scapula, or to cirrhosis of the pancreas, a pancreatic cyst, or

more rarely a growth of the head of the gland, when there will also be progressively deepening, and painless, jaundice. Sometimes the *liver* is found to be enlarged from hyperæmia, fatty infiltration, or cirrhosis, chiefly in gouty or obese subjects who suffer from a mild form of glycosuria.

Respiratory System.—One of the most common complications of diabetes is *pulmonary tuberculosis*, a third to a quarter of all cases dying of this disease. It is often latent, and tuberculosis of the lungs is frequently found post-mortem when the disease has not been recognised as present during life. It may run a rapid course with early excavation, which after death is nearly always found to be much more extensive than would be expected from the symptoms and physical signs. Other pulmonary complications that sometimes occur are *gangrene of the lung*, which also may run a rapidly fatal course, broncho-pneumonia, and acute croupous *pneumonia*, which occasionally gives rise to few subjective signs, but is nearly always fatal.

Circulatory System.—Diabetics of all ages frequently exhibit *arterio-sclerotic changes*, which are, however, comparatively rare in severe cases, and are most commonly met with in the mild, chronic, forms of glycosuria occurring in later life. In the former it is not unlikely that the condition of the vessels is dependent upon the action of organic acids, and other toxic substances, circulating in the blood, while in the latter it is probable that the arterio-sclerosis is antecedent to the glycosuria, at any rate in many instances, and is rather a cause than a complication of the condition. The glycosuria in such cases is most likely dependent upon nutritive changes in the pancreas, and other organs controlling carbohydrate metabolism, arising as a result of the defective blood-supply and the toxæmia that is primarily responsible for the degeneration in the vessel walls. The *heart* is usually not affected in the earlier stages of diabetes, although towards the termination its action may be rapid and feeble, especially when coma is threatening or has developed. In such cases post-mortem examinations show that it is small, and degenerative changes, ascribed by some to the action of circulating acids and toxines, are found in the myo-cardium. The *pulse* is usually regular, and of normal frequency and tension. In some cases, however, it is of high tension, even when there is no kidney mischief, and the patient is under middle age, suggesting that some factor, or internal secretion, producing an effect like epinephrin, is at work.

Renal System.—*Albuminuria* is more especially found in those cases where there is gout, arterio-sclerosis, or obesity. Sometimes

the albumen is, as we have seen, only small in amount and temporarily present, at others it is a sign of intercurrent interstitial nephritis.

Nervous System.—Individuals suffering from persistent glycosuria are subject to nervous affections of the most varied kind. Loss of sexual power is not infrequently an early symptom, and the patient may first seek advice because of *impotence*. A loss of sexual power does not, however, always occur, and in some cases an increase of sexual desire has been observed. In females similar changes have been met with, and *amenorrhœa* is sometimes an early symptom. Conception, pregnancy, and parturition may occur in the normal way in women with diabetes, but there is a great tendency to *abortion*, which, according to Gaudard, occurs in about 30 per cent. of cases. During pregnancy and the puerperal state the disease usually follows a rapidly downward course. *Cramp* in the calf muscles, myalgia, *neuralgic pains* in the distribution of one or more of the spinal nerves, especially the sciatic, are very common in the severer forms of diabetes, and occasionally may be one of the earliest symptoms. The *sciatica* is often bilateral, and the presence of such a condition should always suggest the presence of glycosuria. Next to the sciatic, the trigeminal nerves are most commonly affected. *Multiple neuritis* is met with in some cases. It is usually of a mild type, and most often affects the lower extremities. The arms and legs are apparently never affected together, unlike alcoholic neuritis. Sensory disturbances are, as a rule, most prominent, so that numbness, pain, commonly of a dull aching, gnawing, or burning character, along the nerves, with areas of anæsthesia and hyperæsthesia varying in position and degree, are chiefly found. Only in the severe forms of diabetes are the motor powers much impaired. The condition of *the reflexes* in diabetes has been the subject of numerous researches. Bouchard pointed out that the knee-jerks disappear in about 36 to 37 per cent. of cases. Williamson found that they were absent in half his cases, and that they were lost in a much larger proportion of persons under, than over, thirty years of age. This fact, which at first sight appears astonishing, is no doubt due to the generally greater severity of the disease in young people. An exaggeration of the knee-jerks has been observed by Zaudy and others, particularly just before the onset of coma. A careful investigation of both the cutaneous and deep reflexes was made by Pitres in thirty-six diabetics, and he found that the cutaneous reflexes (abdominal, cremasteric, and plantar) were diminished, or abolished, more often than the knee-jerks, but that the pupillary reflex is

usually intact, a useful distinction from locomotor ataxia. Some of the trophic disturbances, such as atrophy of the skin, herpes, cracking and shedding of the nails, perforating ulcer, &c., have already been referred to.

Affections of the spinal cord, such as tabes dorsalis and disseminated sclerosis, may occasionally precede the appearance of sugar in the urine, but they only very rarely occur as complications of persistent glycosuria.

Cerebral complications, which may cause hæmiplegia or monoplegia, are sometimes encountered. They may arise from hæmorrhage, softening from atheroma of the arteries, or the action of some toxic agent which produces the symptoms without causing any recognisable lesion. Of the *mental change*, depression, irritability, or restlessness, melancholia, with or without suicidal tendencies, and mania are met with, especially in severe cases, when coma is imminent or a very strict diet has been enforced.

Special Senses.—*Ocular* changes are not very common. *Cataract* is the most frequently met with. It is usually double, and soft, and runs a fairly rapid course. It is most commonly seen in severe cases, and is therefore more frequent in young subjects. *Retinitis* and retinal hæmorrhages are occasionally met with, but hardly ever in young persons. A toxic *amblyopia* has been described. *Defective accommodation* is not uncommon, and according to Hirschberg is an early symptom of diabetes. Albuminuric retinitis occurs in cases with complicating kidney mischief. Hæmorrhagic glaucoma, iritis, purulent keratitis, and atrophy of the optic nerve have been seen, although rarely.

Furunculosis of the external *ear*, and rapidly developing otitis media are sometimes met with.

Osseous System.—Fragility of the bones, and delayed union after fracture, have been noticed in severe cases of diabetes. The former probably depends upon the removal of lime salts in an attempt on the part of the organism to neutralise the abnormal acidity of the blood that occurs in such cases, while the latter is part of the general loss of reparative power that is so characteristic of chronic glycosuria.

Anasarca in non-cachectic cases, without albuminuria or any sign of cardiac failure, is occasionally met with. The œdema chiefly affects the legs, and there is pitting on pressure on the skin about the ankles, on the dorsum of the foot, and over the tibia. A slipper or boot will often leave a characteristic impression, which attracts the patient's attention. In a few cases the hands, the face, or other parts are involved. The œdema is believed to depend

upon chloride retention, consequent upon damage of the kidneys, and one certainly finds as a rule an abnormally low chloride excretion in cases where this condition exists.

Acidosis.—It is well known that abstinence from food, or even the sudden withdrawal of carbohydrates from the diet, gives rise to acetonemia, or acetonuria—that is to say, the acetone bodies, including acetone, aceto-acetic acid, and less frequently beta-oxybutyric acid, are excreted in the urine. It is therefore not surprising that in persistent dextrosuria, where the power of utilising dextrose is reduced, and later the general oxidative capacity of the body is interfered with, the acetone bodies and other unoxidised products of metabolism appear in the urine. It would seem probable that the acetone bodies are not abnormal products of metabolism, but that they are normally formed and only accumulate, and are excreted unchanged, when they are not destroyed, owing to some oxidative defect on the part of the tissues. All cases of diabetes, therefore, are not complicated by acetonemia, although it is a constantly present menace, since a deficiency in the oxidative powers of the body is an essential element of the condition. It is only when this reaches a certain stage, however, that the acetone bodies appear in the urine as a necessary consequence.

As we have already seen acetone, aceto-acetic acid, and beta-oxybutyric acid are closely related, and probably have a common source, for although the experiments of Embden, Salomon, and Schmidt suggest that acetone may be derived from substances, such as tyrosine, which do not give rise to oxybutyric acid, it may be taken for granted that when acetone can be detected in the urine by the ordinary qualitative tests, the metabolic powers of the organism are impaired. If acetone alone is present the disturbance is not, as yet, a serious one, for the organism is still capable of oxidising oxybutyric acid to aceto-acetic acid, and of breaking up the latter. Should aceto-acetic acid also be found in the urine, it points to there being a much graver interference with metabolism, for the organism has now not only lost the power of breaking up this substance, but also probably the capacity to oxidise beta-oxybutyric acid, since it is generally found that the two occur together. According to v. Noorden when the acetone in the urine reaches 0·4 to 0·5 grams a day, the perchloride of iron reaction for aceto-acetic acid is invariably obtained, when the acetone reached 0·6 to 1·0 grams a day oxybutyric acid is usually present, when 1·5 grams or more of acetone are met with in the urine beta-oxybutyric acid is rarely absent, although such cases are occasionally met with.

The acetone bodies may also be detected in the internal organs. Geelmuyden found considerable quantities in cases of diabetes that he examined, but there was less in the liver than in the other viscera, and the blood contained less acetone than the urine of the same patient.

The *source of the acetone bodies* was for long a subject of keen controversy, but it now seems to be practically settled that they are principally derived from fat, and particularly from fats containing the lower fatty acids. The chemical experiments of Blumenthal and Neuberg, and of Orgler, have shown that they can also originate from proteins. The observations of Embden, Salomon, and Schmidt on animals prove that they can have this source within the body, but as the first step in the process appears to be the splitting off of the ammonia group of the contained amido acids and their conversion into fatty acids, the immediate antecedent of the acetone bodies formed is in either case the same. It is not now believed, as was at one time thought, that the acetone bodies can be derived from carbohydrates in a similar way to the closely related lactic acid, chiefly because, as Satta showed, the administration of a proper amount of carbohydrate under certain conditions may cause them to disappear from the urine, and that when a patient is put upon a diet that is almost free from carbohydrate they may be eliminated in large quantities.

The place of origin of the acetone bodies is, according to Embden, Salomon, and Schmidt, most probably the liver, although there are some, and notably J. Müller, who considered that they are of intestinal origin. The latter view is not consistent with the observations of Lüthje or v. Noorden, who found that acetonuria is not diminished by the use of laxatives and intestinal antiseptics, but may, on the contrary, increase as a result of the employment of such measures. Many patients suffering from the earlier cerebral symptoms of diabetic coma are, however, peculiarly sensitive to the effects of constipation, and, while the lapse of two or three days without an action of the bowels may greatly exaggerate the symptoms, a free purge is promptly followed by remarkable relief.

It has long been known that diabetic patients are liable to suffer from nervous symptoms, and attacks of dyspnœa, succeeded by coma, which are usually followed by death. Since the coma appears when the quantity of acetone bodies is highest, and is absent when the amount is small, or they are not present, it would seem probable that the condition is dependent upon an excess of these substances in the blood, or upon some condition that is associated with such an excess. The occurrence of coma in diabetes

appears to have been first recorded by v. Dusch in 1854. Three years later Petters discovered what he believed was acetone in the urine, and blood, of a diabetic patient who died with anuria and a subnormal temperature. The presence of this substance in the urine of many cases of diabetes was confirmed by Kaulich, who at the same time pointed out that it is not peculiar to diabetes. In 1874 Rupstein conclusively proved, by an exhaustive chemical analysis of the fluid isolated from the urine of a case of diabetes, that it was really acetone. The same year Kussmaul investigated the toxicity of this substance and found that after large doses had been administered to animals the temperature fell, the pulse became more rapid, and the respirations were shallower. It was therefore assumed that diabetic coma was due to acetone poisoning. It was subsequently shown that a dose of 8 grams per kilo in dogs, corresponding to 500 grams for an adult man, is required to bring about a fatal result, also that acetone can be excreted for years without any symptoms of coma, that in a few cases of coma acetone is absent, that moderate doses do not cause the symptoms described by Kussmaul, 4 grams per kilo in dogs only giving rise to the same effects as ethyl alcohol, and, finally, that the symptoms produced, even by large doses, are not identical with those of diabetic coma.

In 1865 Gerhardt discovered the reaction of diabetic urine with perchloride of iron known by his name, and attributed it to the presence of aceto-acetic ether. Buhl, as the result of experiments performed on rabbits, came to the conclusion that this substance was the cause of diabetic coma. His conclusions were not confirmed, however, by Quincke, who found that aceto-acetic ether is only very slightly toxic for dogs. When it was proved by v. Jaksch that Gerhardt's reaction is in reality dependent upon aceto-acetic acid, it was sought to ascribe diabetic coma to the presence of this substance, but the investigations of Brieger and others proved that it, too, is only slightly toxic.

In 1880 Goetghens compared the acids and the bases in the urine from a diabetic, and found that the latter were in decided excess of what was required to neutralise the known acids present. He consequently suggested that the urine in diabetes contains some unknown acid. Three years later Stadelmann, following the same method, confirmed Goetghens' results, and succeeded in isolating crotonic acid. He suggested that the symptoms of diabetic coma are due to increased acid formation, and that crotonic acid is the immediate cause. The latter conclusion was soon disproved by Minkowski and Külz, who showed that crotonic acid does not exist as such in the urine, but is formed from beta-

o

oxybutyric acid as the result of the chemical manipulation to which it had been subjected. They looked upon the oxybutyric acid as the cause of the symptoms of diabetic coma. The experiments of Waldvogel, Desgrez, and others showed, however, that beta-oxybutyric acid, like acetone and aceto-acetic acid, is only slightly toxic. The theory of oxybutyric acid intoxication was further weakened by the observations of Walther, who proved that symptoms similar to those of diabetic coma can be produced with hydrochloric, or phosphoric, acid. Eppinger showed what disastrous effects the administration of such acids has upon rabbits.

If a rabbit is given repeated small doses of an inorganic acid, which cannot be destroyed by oxidation, the respirations soon become more rapid, the pulse rate is increased, its movements become unsteady, convulsions occur, and stupor, followed by coma, and death supervenes. These manifestations are very characteristic, and particularly the respiratory effects. The animal appears as though it were being asphyxiated (the so-called " air hunger "), yet there is no cyanosis, and the blood is bright red. Analysis of the blood shows that it contains much less carbon dioxide than normal, and that the amount of oxygen is unchanged, but that its alkalinity is diminished. If the urine of such an animal is analysed, it is found to contain increased quantities of the chief inorganic bases, and also of ammonia, indicating the withdrawal of alkalies from the body. Normally the blood carries away the carbon dioxide formed by the tissue, by combining it with the inorganic alkalies it contains. This combination, chiefly bicarbonate of sodium, is decomposed in the lungs, the carbon dioxide escaping, and the carbonate returning to the tissues, to be again converted into bicarbonate. If unoxidisable acids are introduced into the blood, as in the above experiments, they combine with the alkalies, and the blood being thus unable to extract the carbon dioxide, it accumulates in the tissues and the animal consequently succumbs to what has been graphically termed " internal suffocation." This explanation of the effects of acid intoxication is substantiated by the remarkable beneficial effects produced by the intravenous injection of alkalies in many cases. Similar experiments with carnivorous animals, such as dogs, have shown that they are relatively insusceptible to acid intoxication, so that large amounts of acid are required to produce any appreciable effect (Spiro). Analyses of the urine of dogs subjected to this treatment demonstrate that, while the excretion of inorganic alkalies is but little increased, the elimination of ammonia is very much greater than in herbivorous animals. It is consequently believed that a great part of

the acid is, in carnivorous animals, neutralised by ammonia, and that this ammonia is derived from a portion of the nitrogen that normally goes to form urea. As protein metabolism is greater, and more rapid, in carnivora than in herbivora, the ammonia available for the neutralisation of acids is also larger and more readily obtained, so that in the former the inorganic alkalies of the blood are spared for a longer period, and acid intoxication is more difficult to produce. Eppinger has shown that the resistance of rabbits to acid intoxication can be increased by the administration of amino acids, apparently owing to the ammonia that is formed in the metabolism of these substances. Winterberg and Limbeck found that by gradually increasing the dose of acid they could exceed the usual fatal dose for rabbits, and that there was then a larger elimination of ammonia in the urine.

From the close resemblance of the symptoms of diabetic coma to those of experimental acid intoxication, it is now generally agreed that the acetone bodies, or rather beta-oxybutyric acid and to a less extent aceto-acetic acid, produce their effects simply as a result of their acid characters, and not in virtue of any specific poisonous properties. Like the inorganic acids they withdraw alkalies from the body, and as a consequence give rise to '' internal suffocation,'' or '' acidosis '' (Naunyn). In favour of this view is the fact, demonstrated by Orlowski, that titration of the blood in diabetes invariably reveals a reduction in its alkalinity. Minkowski has also shown that the amount of carbon dioxide carried by the venous blood is lowered, from the normal of about 36 per cent., to as little as 3·3 per cent., a reduction comparable only with that seen in extreme cases of experimental acid intoxication, and which was found to be associated with the presence of 46·2 grams of oxybutyric acid in the urine. Further, it is well known that the administration of alkalies will often diminish, or abort, the symptoms of diabetic coma, and that this effect is associated with the elimination of an increased amount of organic acids in the urine, indicating their previous retention within the body, owing to the lack of alkali with which they could combine. It has been calculated that the quantity of native alkali, chiefly sodium carbonate and sodium phosphate, in the entire body is only equivalent to 60 grams of sodium hydroxide. This amount is so small that it would speedily be exhausted by a persistent production of even small amounts of acid, but thanks to the carnivorous habits of man he is able to protect himself against the production of large amounts of acid in virtue of the ammonia that can be derived from the protein of his food. According to v. Noorden, an output of ammonia of

from 4 to 6 grams a day is not at all uncommon in diabetic acidosis, and Stadelmann once found 12 grams. When it is remembered that the normal amount of ammonia in the urine lies between 0·3 and 0·7 grams, according to the diet, and that each gram above that accounted for by the food corresponds to 6·12 grams of oxybutyric acid, it will be seen what a great protection the ammonia is, and what large quantities of acid it can neutralise.

The amount of pathological acid in the urine is most accurately determined by the laborious process adopted by Goetghens of estimating the bases and comparing their total alkali value, expressed in terms of sodium, with the total acid value of the known acids of the urine. By this means the following results were obtained in a healthy person (A), and a case of advanced diabetes with threatening nervous symptoms (B), by Herter :—

Bases.	Grams (A).	Grams (B).	Acids.	Grams (A).	Grams (B).
K_2O	0·9232	2·5540	SO_3 (preformed)	0·9733	0·6857
Na_2O	3·8810	2·4450	SO_3 (combined) .	0·0356	0·1253
CaO	0·2154	0·8035	P_2O_5 (bibasic) .	0·7432	0·8521
MgO	0·0806	0·1973	P_2O_5 (monobasic)	0·1987	0·1756
$N(NH_3)$ . . .	0·4707	3·1130	Uric acid . . .	0·0584	0·0271
			Cl	4·4760	1·5110
Total bases . .	5·5709	9·1128	Total acids . .	6·4852	3·3768

In the healthy person the total acids exceeded the total basis in this instance by 0·9143 gram, but in the diabetic there was an excess of bases over acids which equalled 5·736 grams. In the former case the apparent excess of acid was due to the presence of some organic base with which the acid was united, while in the latter the excess of base must correspond to some organic acid not allowed for in the estimation. Assuming that the acid was beta-oxybutyric acid, this amount of sodium would correspond to about 26 grams. A rough, and for clinical purposes sufficient, index of the degree of acidosis can, however, be obtained by estimating the daily excretion of ammonia, for, as a rule, ammonia is the base that is chiefly increased in diabetes. The acetone, aceto-acetic, and oxybutyric acid may be separately estimated by the methods already described, but the processes involved are too lengthy for routine work.

The explanation of diabetic coma by the theory of an acid

intoxication due to the formation of beta-oxybutyric, and aceto-
acetic, acid is at present widely held, but why these acids should
be formed is as much a puzzle as ever. The statement that the
body has lost its oxidative powers is, after all, only a cloak for
our ignorance on the matter. It has been pointed out by Kraus,
Rumpf, and others that in a few cases of diabetes coma develops
without any increase in the elimination of organic acids in the urine,
and that alkali therapy has not always the remedial effect that
might be expected if the coma were always a result of acidosis.
Moreover, cases have been reported by v. Noorden and other ob-
servers in which large amounts of acetone (5 to 6 grams) and oxy-
butyric acid (30 to 40 grams) have been excreted in the urine,
and yet the patient has lived comfortably for years. It has been
suggested by Naunyn that the coma in cases without increased
acid formation, may be due to the presence of some unknown
toxine which exerts a direct action on the cerebral cells, and
especially those of the respiratory centre. Klemperer holds the
view that both the coma and the abnormal production of acid
in diabetes are due to the presence of some such toxine. Labbé
maintains that while acidosis and diabetic coma are closely related
they are probably due to different mechanisms, and points out
that many features of coma indicate an intoxication with poly-
peptides. It has been pointed out by Porges that, since the
amount of carbon dioxide in diabetic blood is abnormally low, and
it is known that this substance is of importance in relation to the
activity of the heart, it is possible that several of the symptoms
of coma result from the lowered carbon dioxide tension. Although
the diversity of the symptoms met with in different cases of
diabetic coma suggests that more than one cause may be operative,
these explanations belong rather to the realm of theory than of
fact, at any rate at present.

Symptoms of Diabetic Coma.—Coma is one of the most frequent
and serious symptoms of diabetes, but is more common in persons
under, than over, forty. It is not necessarily a late symptom,
but may occur apparently early in the course of the disease. The
clinical symptoms are, however, always preceded by the chemical
signs of acidosis ; for, in the large majority of cases at least, coma is
but the culminating point of an acid intoxication. It is therefore
of the utmost importance that the urines of all diabetics should
be watched for evidence of this condition. This is true not only
of the clinically severe types, but also of the milder forms, since
fatigue, anxiety, complicating and intercurrent diseases of various
kinds, alcoholic intoxication, general anæsthesia, and complete

abstinence from carbohydrate food are all liable to rapidly develop the acidosis and bring about a condition of severe intoxication, with its attendant danger of coma, in any case where abnormal acid formation has commenced.

In the majority of cases the onset of the coma is more or less sudden, often coming on without any apparent cause. In some there may be prodromal symptoms extending over several days, or, occasionally, weeks. The most common of these are loss of appetite, and obstinate constipation. When a diabetic complains of an impairment of appetite and gastric disturbances after every meal, it is always a grave sign, especially if the bowels are not being freely opened, and the liver is found to be enlarged. According to Lépine acceleration of the pulse is often an early, although not a characteristic, symptom. Lassitude and apathy, or mental irritability and restlessness, with, maybe, epigastric pain and vomiting, are often the first indications that are noticed. The patient soon becomes drowsy, and the drowsiness gradually verges into coma. At first it is possible to rouse him, but this becomes more and more difficult. Meanwhile the respirations, although regular, are much increased in range, and inspiration is prolonged, so that the breathing is often of a sighing character, a condition usually described as " air-hunger." The breath has a characteristic sweet, fruity, smell, that often pervades the whole room, and, with the character of the respirations, at once suggests to an experienced observer the cause of the coma, even if the patient has not previously been seen. The action of the heart is rapid and weak, the features become drawn. The eyes are half closed, the pupils are sometimes dilated, sometimes contracted, but they generally react well to light until deep coma sets in. The extremities are cold, and are often cyanosed. The temperature, which at the onset may have been temporarily raised, is subnormal, although it may again rise just before death takes place. Twitchings and convulsions occasionally occur. The urine is generally much diminished in quantity, and is markedly acid in reaction. Acetone and aceto-acetic acid, although usually present, are not so abundant as before, but the amount of beta-oxybutyric acid is generally much increased. The total output of nitrogen is increased relative to the sugar, but the proportion of nitrogen excreted in the form of urea is diminished, while the ammonia nitrogen is correspondingly increased, but this too is in some instances diminished. The urine nearly always contains albumen, and numerous granular and hyaline casts are seen on microscopical examination of the deposit. When coma is fully developed death almost invariably

follows, and usually takes place in from twelve to forty-eight hours after the onset of the characteristic symptoms.

Beside the preceding, which may be termed the " dyspnœal form of diabetic coma, there is another type in which sudden collapse, probably from failure of the heart, occurs soon after the drowsiness and coma have developed, and yet a third in which a period of acute excitement and ataxia, suggestive of acute alcoholic intoxication, precedes the coma. In a few instances, where death occurs from coma in diabetes, it is clearly due to the ordinary causes, such as uræmic intoxication, &c., but in these the character of the symptoms, and the presence of definite renal, or other, complications usually suffice to differentiate the condition from true diabetic coma.

BIBLIOGRAPHY

Abderhalden, *Zeit. f. phys. Chem.*, xliv.

Barth and Autenrieth, *Hoppe-Seyler's Zeit.*, 1902.

Biscofswerder, *Inaug. Dissert.*, Berlin, 1896.

Blumenthal and Neuberg, *Deut. med. Woch.*, 1901.

Boecker, *Deut. Klinik.*, 1853.

Bose, *Brit. Med. Journ.*, 1907.

Brandenburg, *Zeit. f. klin. Med.*, xlv.

Bremer, *New York Med. Journ.*, lxiii., lxvi. ; *Cent. f. inn. Med.*, 1897.

Buhl, *Zeit. f. Biol.*, 1880.

Burian and Schur, *Pflüger's Arch.*, lxxx., xciv.

Busse, *Münch. med. Woch.*, 1901.

Cammidge, *Lancet*, 1904, 1909.

Davies, *Lancet*, 1909.

Desgrez, *Compt. Rend. d. l. Soc. d. Biol.*, 1907.

Dickinson, *Diseases of the Kidneys*, 1875.

Downes and O'Brien, *Intercol. Med. Journ. of Australia*, 1909.

Von Dusch, *Zeit. f. ration. Med.*, 1854.

Ebstein, *Virchow's Arch.*, 1899.

Embden, Salomon, and Schmidt, *Hofmeister's Beiträge*, 1906.

Embden and Schliep, *Centralb. f. Path. u. Pharm.*, 1907.

Eppinger, *Wiener klin. Woch.*, 1906 ; *Zeit. f. exp. Path. u. Pharm.*, 1906.

Ewing, *Clinical Diagnosis of the Blood*, 1904.

Fischer, *Virchow's Arch.*, 1903.

Frerichs, *Diabetes Mellitus*, 1884.

Folin, *Harvey Lectures*, 1908.

Funck, *Deut. med. Woch.*, 1911.

Futterer, *Verh. d. pl. med. Gesell.*, 1888.

Gabritschewsky, *Arch. f. exp. Path.*, xxviii.

Geelmuyden, *Zeit. f. phys. Chem.*, 1904.

Gerhardt, *Wiener med. Presse*, 1865.

Goetghens, *Zeit. f. phys. Chem.*, 1885.

Grübe, *Münch. med. Woch.*, 1895.

Habershon, *Journ. of Path. and Bact.*, 1906.

Henriques, *Zeit. f. phys. Chem.*, xxvi.

Herrick, *Amer. Journ. of Med. Sci.*, 1900.

Herter, *Lectures on Chemical Pathology*, 1902.

Hoppe-Seyler, *Physiol. Chem.*, 1880.

Jacoby, *Zeit. f. klin. Med.*, 1897.

James, *Edin. Med. Journ.*, 1896.

Jolles and Winkler, *Arch. f. exp. Path. u. Pharm.*, 1900.

Kaulich, *Prag. Vierteljah.*, 1860.

Kolisch, *Wiener klin. Woch.*, 1897.

Kraus, *Zeit. f. Heilkunde*, x.

Külz, *Zeit. f. Biol.*, 1884.

Kussmaul, *Deut. Arch. f. klin. Med.*, 1874.

Labbé, *Presse Médicale*, 1912.

Lécorché, *Le Diabète chez les femmes*, 1886.

Leichenstern, *Untersuch. u. d. Hœmoglob.*, 1878.

Lépine, *Diabète Sucré*, 1909.

Loch, *Zentralb. f. inn. Med.*, 1905.

Locke, *Boston Med. and Surg. Journ.*, 1902.

Lüthje, quot. Neuberg and Blumenthal, *Deut. Arch. f. klin. Med.*, 1903.

Luzzato, *Festsch. z. v. Salkowski*, 1904.

Maguire, Fowler's *Dict. of Med.*, 1890.

Mayer, *Zeit. f. klin. Med.*, 1901.

Mendal and Rose, *Journ. of Biol. Chem.*, 1911.

Mies, *Münch. med. Woch.*, 1894.

Minkowski, *Arch. f. exp. Path.*, 1884 ; *Berl. klin. Woch.*, 1892.

Möhr, *Zeit. f. klin. Med.*, 1901 ; *Deut. med. Woch.*, 1905 ; *Zeit. f. exp. Path. u. Therap.*, ii.

Moraczewski, *Zeit. f. klin. Med.*, 1904.

Naunyn, Nothnagel's *Spec. Path.*, vii. 6.

Naunyn and Reiss, *Reichert. u. Dubois Arch.*, 1869.

Neisser and Derlin, *Zeit. f. klin. Med.*, 1904.

Neubauer, *Journ. f. prakt. Chem.*, lxvii.

Neumann and Mayer, *Zeit. f. phys. Chem.*, 1903.

Nicola, *Gioin d. Roy. Acad. d. mid.*, 1904.

Von Noorden, *Die Zuckerkrank*, 1901 ; *Twentieth Cent. Practice*, ii. 99 ; *Handb. d. Ernahrungstherapie*, 1904.

Orgler, *Hofmeister's Beitr.*, 1901–2.

Orlowski, *Centralb. f. Stoffwechsel*, 1902.

Pavy, *Lancet*, 1878.

Petters, *Prager. Vierteljahr*, 1857.

Porges, *Wien. klin. Woch.*, 1911.

Quincke, *Berl. klin. Woch.*, 1880.

Redier, *Stomatologie*, 1909.

Rumpf, *Berl. klin. Woch.*, 1895.

Rupstein, *Centralb. F. d. Med. Wissensch.*, 1874.

Satta, *Hofmeister's Beitr.*, 1905.

Schmitz, *Berl. klin. Woch.*, 1891.

Schneider, *Münch. med. Woch.*, 1899.

Seegen, *Diabetes Mellitus*, 1893.

Stadelmann, *Arch. f. exp. Path.*, 1883.

Strauss, *Die Chron. Nierenentzund*, 1902.

Tessier, *Thése de Paris*, 1876.

Tenbaum, *Zeit. f. Biol.*, 1896.

Tirard, *Lancet*, 1909.

Waldvogel, *Die Acetonkörper*, 1903.

Waterman, *New York Med. Record*, 1882.

Williamson, *Diabetes Mellitus*, 1898 ; *Med. Chronicle*, 1909.

CHAPTER VII

In some cases persistent dextrosuria is undoubtedly associated with pathological changes in the pancreas, which can be demonstrated after death by the naked eye or by the microscope. In others no pancreatic lesion can be discovered, and, while it is possible that, as v. Noorden has suggested, severe disturbances of the chemical functions of an organ are not necessarily associated with recognisable changes in its anatomical structure, it is probable that in such cases the primary lesion is in some other organ. The work of Falta, and others, on the influence exerted by the ductless glands on carbohydrate metabolism suggests, as we have seen when considering experimental glycosuria, that affections of the thyroid, pituitary, and supra-renal glands play a part in the pathology of diabetes, and that it is to them we must look for the primary cause, at least in some instances. The nervous theory of diabetes that so long held the field has proved not to be universally true, but it is quite clear that lesions, or disturbances of function, of the brain, spinal cord, &c., may also give rise to persistent glycosuria. It is therefore necessary that, in the first place, we should consider what morbid changes have been met with in these various structures in diabetes.

The Pancreas.—The pancreatic origin of certain cases of diabetes appears to have been suspected by pathological anatomists and clinicians long before a definite pancreatic theory of diabetes was propounded. As far back as 1788 Cowley described a case in which glycosuria was associated with disease of the pancreas. The patient was a very stout man of thirty-five with an alcoholic history ; post-mortem the pancreas was found to be atrophied, and numerous calculi were present in the ducts. Chopart published a similar case in 1821, and Bright, in 1833, gave an account of a diabetic of nineteen with jaundice and fatty stools, whose pancreas was found post-mortem to be atrophied, and to contain a hard nodular tumour in the head, which was firmly adherent to the duodenum. Isolated examples of similar conditions were subsequently published by other observers with increasing frequency.

The first to definitely suggest a causal relationship between disease of the pancreas and diabetes in some cases was Bouchardat, in 1846. He based his belief, however, on the view that the glycosuria was dependent upon alterations in the digestive functions of the gland. In his *Traité du Diabète*, published in 1875, he said : '' Si j'ai observé, chez quelques glycosuriques, une altération bien manifeste du pancréas ou de ses conduits, il est d'autres observateurs (et je suis moi-même de ce nombre) qui, pour la plus grande majorité de cas, n'ont rien trouvé d'anormal dans le pancréas des glycosuriques,'' a statement which holds good to the present day. In 1877 Lancereaux, basing his conclusions on the literature and two cases of his own, confirmed Bouchardat's conclusions, but sought to distinguish a special type, which he termed '' diabète maigre,'' characterised by profound wasting and a rapid course, as being characteristic of serious alterations in the structure of the pancreas. The fallacy of this distinction was, however, proved by subsequent observers, who showed that the glycosuria accompanying disease of the pancreas is not associated with any particular symptoms, and that '' diabète maigre '' may occur without there being serious structural changes in the gland.

The experimental work of von Mehring and Minkowski, published in 1889, established the pancreatic theory of diabetes on a secure footing, for it proved that extirpation of the gland in animals gives rise to symptoms more nearly resembling those met with in severe human diabetes than can be produced by any other means, and also showed that the influence the pancreas exerts on carbohydrate metabolism is independent alike of the external secretion of the gland, and of its nervous connections. These observations aroused fresh interest in the condition of the pancreas in diabetes, and a number of observers published statistics bearing on the point. Among the earliest of these were the investigations of Windle, who reported that in 139 cases of diabetes the pancreas had been found to be diseased in 74, or 53 per cent. Seegen, however, who analysed the records of 92 cases stated that a pancreatic lesion had been noticed in only 17 (19 per cent.), while Frerichs found disease of the pancreas in 16 out of 44 cases of diabetes (36 per cent.). Hansemann again reported a much higher percentage of pancreatic lesions, 40 out of 54 (74 per cent.) in the cases of diabetes examined after death in the Berlin Pathological Institute. Bloch collected 22 cases from the records of the Vienna General Hospital, and found that in 12 (55 per cent.) the pancreas had been recognised as abnormal. Oser quotes 42 cases with pancreatic lesions in 161 diabetics (26 per cent.). Williamson, in his work on *Diabetes*

Mellitus, published in 1898, gives an account of 23 cases in which special attention was paid to the condition of the pancreas, and states that in 15 of these (79 per cent.) there was evidence of disease. These widely divergent results are no doubt, to a great extent, to be explained by a difference of opinion as to what is to be regarded as normal and what as pathological, and it is apparent from a study of the published records that the more carefully and systematically disease of the pancreas has been searched for in cases of diabetes, the more frequently has it been found.

The use of the microscope by recent observers has considerably increased the proportion of cases in which lesions of the pancreas have been discovered in association with diabetes, and this has been particularly marked since attention was drawn to the possible relationship of the islands of Langerhans to the internal secretion of the gland. Opie, who made a histological examination of the pancreas in 19 cases, found some abnormality in 15 (79 per cent.), and in 4 of these it was not until they were submitted to microscopical examination that a lesion was discovered. Bosanquet, using the microscope, records disease of the pancreas in 17 out of 19 cases (90 per cent.) that he investigated. Hansemann has recently claimed that every case of '' true '' diabetes is associated with demonstrable changes in the pancreas, if the gland is examined quite fresh and before any auto-digestion has taken place.

According to the older observers the most common lesion of the pancreas met with in diabetes is *atrophy* of the gland. Windle found it in over 59 per cent. of the cases he examined, and Frerichs in 75 per cent. The statistics quoted by Hansemann from the Berlin hospitals in the space of ten years, show 49 cases of diabetes with disease of the pancreas, in 36 (90 per cent.) of which there was simple atrophy, and in 3 (8 per cent.) atrophy and sclerosis. The more recent observations of Williamson and Opie give much lower figures, the former finding simple atrophy in 4 out of 11 cases (27 per cent.), and the latter in 4 out of 15 (26 per cent.). Some explanation of this difference is afforded by the more exact methods of observation employed by the modern observers, and there can be no doubt that in the past too great reliance on the naked-eye characters caused many cases to be classified as simple atrophy which in reality were examples of atrophic changes resulting from chronic inflammation of the gland.

In a few cases *fatty degeneration* of the pancreas has been found after death as the only discoverable lesion. Bosanquet met with a recognisable degree of fatty change in 10 out of 100 cases, which in 3 was combined with some fibrosis. Williamson in his series

found one case of lipomatosis in which there was atrophy and fatty degeneration, and one where, beside atrophy and fatty degeneration, there was evidence of inflammatory change.

The earliest recorded case in which disease of the pancreas was found to be associated with diabetes was, as we have seen, one of *pancreatic calculi.* Hansemann, however, was only able to find fourteen instances in 72 cases (19 per cent.) collected from the literature, and Oser quotes but twenty-four examples in 188 cases of diabetes (14 per cent.), so that the association is not very common, particularly as the lesion is so obvious that it would not be readily overlooked. The mere presence of calculi cannot be regarded as directly responsible for the diabetes, since blocking of the ducts by ligature, or otherwise, has been proved not to cause glycosuria. It is to the fibrotic changes accompanying them that we must therefore look for the explanation. That this is so is shown by the fact that diabetes is only found in cases where there is very marked overgrowth of fibrous tissue, whereas in those instances where the concretions are not associated with advanced interstitial changes sugar does not appear in the urine.

In a similar way although *cysts* of the pancreas have been met with in from 5 per cent. (Oser) to 7 per cent. (Dieckhoff) of diabetics showing pancreatic lesions, there are many cases of cysts in which glycosuria does not occur. In some instances sugar may appear in the urine some time after a cyst has been surgically treated, owing probably to the advance of the chronic inflammatory changes to which the formation of the cyst was originally due. A case of this description, under the care of Dr. Churton, was operated on by Mr. Mayo Robson in June 1896.

At the time of the operation the urine was free from sugar and showed no other abnormality, save that it gave a well-marked '' pancreatic '' reaction. In February 1905, I was able to obtain a twenty-four hours' sample of urine, and a specimen of the fæces, from this case. The former measured 62 oz. and had a sp. gr. of 1·030. It reduced Fehling's solution, and gave characteristic glucosazone crystals with phenylhydrazin. Quantitatively 4·5 per cent. of sugar (80 grams in the twenty-four hours) was found. Aceto-acetic acid was absent, but there was a trace of acetone. There was no albumen, bile-pigment, urobilin, or indican. Bial's pentose reaction was negative. The total nitrogen, urea, uric acid, chlorides, phosphates, and sulphates were found to be normal in amount, but oxalates were in excess (0·32 grams in the twenty-four hours). This specimen of urine also gave a '' pancreatic '' reaction. The fæces were light yellow in colour, and were faintly alkaline in reaction. There was no marked excess of fat, but the normal relation between the unsaponified and

saponified fats was disturbed, the former constituting 15 per cent., and the latter only 5 per cent. of the dry weight, thus pointing to there being some interference with the digestive functions of the pancreas.

The association of *cancer* of the pancreas with diabetes is relatively uncommon. Windle found it in 4 per cent. of his cases, Frerichs in 6 per cent., Dieckhoff in 7 per cent., Heiberg in 3 per cent., and Williamson once in a series of twenty-three consecutive cases. Glycosuria has been met with in 6 per cent. of the cases of primary malignant disease of the pancreas that I have examined, and once where the gland was involved in a secondary growth. The latter is of particular interest, for it demonstrated very clearly the importance of the pancreas in carbohydrate metabolism in the human subject, and also the value of the " pancreatic " reaction in diagnosis.

The patient was first seen in December 1905 ; there was then an abdominal tumour which was suspected to be pancreatic, but an examination of the urine gave no " pancreatic " reaction, and there was also at that time no sugar. An exploratory examination was performed by Mr. Mayo Robson, and a growth was found in the first part of the duodenum, but quite free from the pancreas. On January 18 a second specimen of urine was examined, and found to be free from sugar, but it gave a well-marked " pancreatic " reaction, suggesting that the pancreas was then involved in the disease. At the request of the patient's friends the abdomen was re-opened a few days later, and it was then found that the growth had invaded the pancreas, as had been suspected. In the early part of May 1906, examination of the urine showed 5·25 per cent. of sugar, and a modified " pancreatic " reaction gave many fine crystals soluble in 33 per cent. sulphuric acid in five to ten seconds. A month later the sugar had increased to 7 per cent., and a much less marked " pancreatic " reaction was obtained. In July the urine contained 7·25 per cent. of sugar, and the " pancreatic " reaction gave only a few crystals. In August, 7·5 per cent. of sugar was present, and no crystals were found on carrying out the modified " pancreatic " test. In October the urine contained 9·5 per cent. of sugar, and the " pancreatic " reaction was negative. In spite of the high percentage of sugar in the urine, the general condition of the patient remained fairly satisfactory, and she complained of no other symptoms than thirst and a voracious appetite. Considerable quantities of acetone and aceto-acetic acid were found in the urine in May, but with careful treatment they gradually diminished in amount, until in the early part of October only traces could be detected. Towards the end of October the gall-bladder was discovered to be distended, and a few days later jaundice developed. The patient died deeply jaundiced on November 5th.

In some cases of malignant disease of the pancreas glycosuria

has appeared as an early symptom, and has later disappeared, while in others it has only been met with toward the termination of the disease. The temporary glycosuria is probably to be explained by a transitory disturbance in the functions of the gland, caused by an inflammatory reaction consequent on the spread of the growth. It has also to be borne in mind that when a portion of the pancreas has been destroyed, whether by growth, or as the result of chronic inflammatory changes, the condition resembles that produced in animals by partial extirpation of the gland, so that if carbohydrates are excluded from the diet, or are much reduced, an alimentary glycosuria that previously existed may disappear. In most recorded cases where sugar has appeared in the urine as a terminal symptom, either the whole organ has been replaced by a mass of growth, or the portions that have remained have undergone sclerotic changes, so that no normal pancreatic tissue has been left to carry on the functions of the gland.

The absence of permanent diabetes in most cases of cancer of the pancreas is due to the growth being limited, in many instances, to one portion of the gland, generally the head. In about 29 per cent. of cases, however, this explanation will not hold good, for in about that proportion there is a diffuse growth affecting the whole organ. It is supposed that in these cases either the tumour cells possess the same secretory functions as the normal gland tissue, or that the new growth insinuates itself between the pancreatic cells in such a way as to obliterate the normal structure of the organ without destroying it entirely. That such a process of growth is possible is shown by the presence, in some instances, of unaltered islands of Langerhans in the midst of the cancerous material, while in support of the former hypothesis Hansemann points out that in primary carcinoma of the supra-renals Addison's disease is rare. Lépine and Heiberg have both reported cases in which cancer of the pancreas occurred in diabetics who had passed sugar in their urine for several years previous to the onset of the symptoms of malignant disease. Such cases rather favour the view advanced by some, that carcinoma of the pancreas may originate in groups of cells isolated by fibrosis of the gland in much the same way as primary cancer of the liver appears to arise from groups of cells similarly isolated in cirrhosis of that organ.

Inflammatory lesions of the pancreas and their sequelæ are by far the commonest pathological changes affecting the gland, but until recently they have failed to receive the recognition their importance deserves. It is consequently not surprising to find that the association of diabetes with pancreatitis and its results,

has been, to a great extent, overlooked, or that the condition has been referred to some other cause. Calculi and cysts, as we have seen, are not of themselves responsible for the glycosuria with which they may be associated, but occur in the course of a chronic inflammation which ultimately destroys the structure of the gland ; the special form of atrophy of the pancreas described by Hansemann as common in diabetes is in reality a fibrosis due to chronic inflammatory changes, while the diabetes associated with some cases of malignant disease is apparently brought about by a secondary inflammation set up by the presence of the growth. Dieckhoff in his analysis of fifty-three cases found acute pancreatitis in 10 per cent., and chronic pancreatitis in 36 per cent. Williamson met with four instances of cirrhosis of the pancreas in twenty-three cases, and Opie with four of chronic inflammation in nineteen cases, so that inflammatory change probably plays a not unimportant part in the production of diabetes, especially if the secondary manifestations to which reference has been made are taken into account.

Acute pancreatitis is not a common disease, and for this reason alone is not frequently met with as a cause of diabetes. In 188 cases collected by Oser there were three in which glycosuria was associated with hæmorrhage into the pancreas, three with necrosis of the gland, and six with abscess. In 100 cases of acute inflammation collected by Fitz and by Sietz glycosuria was present in two. The reason for the comparative rarity with which glycosuria occurs in acute pancreatitis appears to be that when the whole organ is destroyed death usually follows very rapidly, and when the progress of the disease is less acute portions of the gland are left unaffected. The experiments of Guleke on dogs have shown that when complete necrosis of the pancreas has been induced, by injecting oil into the ligatured pancreatic duct, glycosuria always occurs, but that when a portion of the pancreas has been left intact no sugar is found in the urine. A fatal case of hæmorrhagic pancreatitis with destruction of the whole gland and associated with the appearance of sugar in the urine was described by Bosanquet in his Goulstonian lectures.

The patient, a laundress aged fifty-three, was under the care of Dr. J. M. Bruce in Charing Cross Hospital. A week before admission she was seized with acute pain in the abdomen, which rapidly swelled and became hard to the touch. She had previously had no symptoms of diabetes, but now complained of thirst, and on examining the urine it was found to contain from 10·2 to 11·25 grains of sugar in the twenty-four hours. Her temperature rose, and she had rigors on two successive days. Finally she died collapsed, but without any symptoms

of coma. At the necropsy a breaking-down mass, with much bloody fluid, was found in the situation of the pancreas. There was also diffuse fat necrosis and evidence of recent peritonitis.

Such a case constitutes a natural experiment on the removal of the pancreas in a human being and, as Bosanquet points out, the results exactly corresponded with those obtained in animals.

Benda and Stadelmann found 3 to 5 per cent. of sugar in the urine of a patient who succumbed to hæmorrhagic pancreatitis with fat necrosis, in whose previous history there was nothing to suggest antecedent diabetes. In a few instances acute or subacute pancreatitis has been recovered from, but left the patient with persistent glycosuria. Gifford Nash's case already referred to is an example of this, for, although the sugar that appeared in the urine during convalescence disappeared for some six or seven months, a permanent glycosuria was ultimately established. Brentano has described a case of necrosis and sloughing of the pancreas in which a sub-diaphragmatic abscess was opened. The patient eventually recovered, but left the hospital with a pancreatic fistula and persistent glycosuria.

Chronic interstitial pancreatitis can be divided histologically into two types—(a) *an interlobular form*, in which the overgrowth of fibrous tissues takes place chiefly between the lobules, and (b) *an interacinar form*, where the newly formed fibrous tissue is diffusely distributed within the lobules and between the individual acini. According to Opie, the former is rarely found to be associated with diabetes, while glycosuria is a very much more common symptom of the latter. To the first type belong the chronic inflammatory changes that result from obstruction of the pancreatic duct, gall-stones, &c. The second variety is of unknown origin, but its very constant association with arterio-sclerosis suggests that they have a common cause, possibly an intestinal toxine. Pancreatitis of the interlobular type was found by Opie to be associated with glycosuria in only one out of twenty-nine cases, but seven out of nine cases with interacinar pancreatitis had suffered from diabetes. I have had considerable experience of the glycosuria following symptoms of pancreatic disease and gall-stones, and have already summarised the findings in 200 cases of diabetes that have come under my observation. In another series of sixty-five consecutive cases where biliary calculi were discovered in the common bile duct at operation, and the pancreas was enlarged and hard, I was able to detect sugar in the urines of only four (16 per cent.). The quantity was very small in all of them, under 0·2 per cent. in three, and 0·4 per cent. in the fourth. After operation no sugar could be found

P

in the urine of the former, but it was still present in the fourth case, where it slowly increased in amount. This patient died of diabetic coma, I am informed, nineteen months after the operation. I have met with interacinar pancreatitis in three cases of diabetes which I have had the opportunity of examining after death, and have found interlobular pancreatitis to be the lesion present in six gall-stone cases I have investigated histologically. In 220 cases of diabetes collected by Windle gall-stones were present in only one (0·45 per cent.). In another series of 142 cases of diabetes collected by Williamson, biliary calculi had been also found in only one of them (0·7 per cent.). Rolleston states that in a consecutive series of twenty-seven cases of diabetes examined at St. George's Hospital gall-stones were present in four, and that in two of these the calculi were in the common bile duct, and were associated with chronic interstitial pancreatitis.

One great stumbling-block in the way of the ready acceptance of the pancreatic theory of diabetes has been the very frequent occurrence of lesions, and often very marked lesions, of the pancreas without glycosuria; but if we accept the view that the islands of Langerhans are concerned in the elaboration of the hypothetical internal secretion, and that so long as they are intact carbohydrate metabolism will not be interfered with, this difficulty is met, for experiments upon animals, and observations on the human subject, have shown that fibrosis of the gland, fatty degeneration, &c., may involve the secreting parenchyma to a remarkable degree and yet leave the cell-islets unaffected.

The very frequent occurrence of diabetes in interacinar pancreatitis, and its comparative rarity with the interlobular form, is considered by Opie to depend upon the relationship of the fibrous tissue overgrowth to the cell-islets; for, owing to the diffuse distribution of the fibrous tissue in the former, the islands are affected at the same time as the other elements of the gland, while in interlobular pancreatitis the proliferating fibrous tissues invades the lobules from the periphery, so that the cell-islets only suffer when the process is far advanced and the secreting parenchyma has been largely replaced by masses of scar-tissue. Opie's case of interlobular pancreatitis mentioned above, in which glycosuria was present, showed far-advanced induration with fibrosis of the islands of Langerhans, and the two cases of interacinar pancreatitis in which diabetes was absent were both found to be in an early stage of the disease, so that the cell-islets were unaffected.

Schäfer in 1895 was the first to suggest that pathological changes in the islands of Langerhans might be the cause of diabetes. In

1900 Ssobolew announced that the cell-islets were absent in two cases of diabetes that he examined. The same, and the following year, Opie published accounts of marked lesions of the islands of Langerhans in cases of diabetes investigated by him. The latter stated that he had met with hyaline degeneration of the islands of Langerhans in seven out of nineteen cases (35 per cent.) of diabetes, that in three the condition of the cell-islets was such as to render them almost completely functionless, though the parenchyma was relatively well preserved, in three others the lesion of the cell-islets appeared to be less widespread ; while in another with severe diabetes the islets were so altered as to be completely unrecognisable, and the secreting parenchyma was in great part destroyed. Opie's striking results aroused fresh interest in the subject, and stimulated a more searching inquiry into the condition of the islands of Langerhans in fatal cases coming to autopsy. Other observers have since described hyaline degeneration of the islands of Langerhans in diabetes, but it would not appear to be by any means as common as Opie's experience suggests, and the possibility of the degeneration being a secondary change has also not been excluded. Bosanquet has reported a case in which he found it apart from diabetes, in association with extensive arterio-sclerosis of the pancreatic vessels, in a woman who died after an operation for gall-stones. According to Weichselbaum and Stengel, who studied the islands of Langerhans in thirty-five cases of diabetes, the lesion most frequently met with is simple atrophy of the cells, with vacuolisation and liquefaction of the protoplasm. Sclerosis of the cell-islets was met with in four out of their second series of seventeen cases. As the result of an investigation of a further series of 183 cases, Weichselbaum came to the conclusion that the changes in the islands of Langerhans are constant and peculiar to diabetes, and that they may be classified under four headings—(1) Dropsical degeneration, in which the epithelial cells become translucent, while some are destroyed ; there may be small-celled infiltration of the capsule with hypertrophy of the connective tissue, and consequent atrophy and disappearance of the epithelial cells of the islands ; this lesion is seen in subjects under forty, and is never associated with arterio-sclerosis. (2) Sclerosis of the islands with hypertrophy of the surrounding connective tissue and chronic interstitial pancreatitis, often accompanied by fatty change ; it is seen in persons over fifty, and is always associated with arterio-sclerosis. (3) Hyaline degeneration of the sheaths of the vessels with consequent compression of the cells of the islands ; it is seen in advanced age, and is associated with sclerosis of the

pancreatic vessels ; this form is often combined with the second.
(4) Hæmorrhage into the islands, which may occur apart from
diabetes ; regeneration or hypertrophy of the islands often ensues.
Weichselbaum did not find analogous lesions in any other disease,
acute or chronic ; he accounts for the fact that many have failed
to notice them on the ground that often dropsical degeneration
only is present, which may easily pass unperceived, and that the
lesions may be restricted to portions only of the pancreas. Herzog
studied three cases of diabetes in which the cell-islets were the
seat of marked sclerotic changes, and Schmidt has met with two
in which there was advanced interacinar pancreatitis, so seriously
involving the islands of Langerhans that they were converted into
fibrous tissue balls resembling fibrosed glomeruli. In a case re-
ported by Lépine the islands were surrounded, and in places partly
destroyed, by a new growth of fibrous tissue. Gentes has also
described a case of diabetes with chronic interstitial pancreatitis
invading the islands of Langerhans. An acute inflammation,
limited to the cell-islets, was met with by Schmidt in the case of
a child of ten whose urine contained 6·8 per cent. of sugar. Focal
necrosis of the pancreas involving the islands of Langerhans has
been described by Opie in one case. Absence of the islands, or a
diminution in their number, has been reported by several observers ;
but as Opie has pointed out, it is necessary that the sections of
diseased pancreas should be compared with preparations from
corresponding parts of a healthy gland before any conclusion is
drawn as to the absence or diminution of the cell-islets, for their
distribution varies very much in different parts, being very numerous
in some situations, particularly the tail, and almost absent in
others. Opie compared the size and distribution of the cell-islets
in the head, body, and tail of the pancreas in eight cases of diabetes,
and found that the figures obtained showed no constant departure
from the normal. A striking diminution in the number of islets
was, however, seen in two other cases, and in one of these, a child
of fourteen, the diabetes was hereditary, suggesting that it might
be due to a congenital defect of the gland.

Dale and others, as we have seen, deny, on histological grounds,
that the islands of Langerhans are independent structures, and that
they therefore have any particular function in carbohydrate
metabolism. Some observers have come to a similar conclusion
as the result of pathological investigations. The chief of these
has been Hansemann, who investigated thirty-four cases of diabetes,
and found that cell-islets were present in all. He states that in
some, where nearly the whole parenchyma had been destroyed by

fat, or interstitial fibrosis, they were diminished in number but were structurally unchanged. In six cases he found that the islands were invaded by what he regarded as hyaline connective tissue, but since they were not all affected, and there was an accompanying fibrosis of the gland, he considered that it was a matter of chance as to whether the islands were involved or not, although he admits that he has not met with a case in which fibrosis affected the cell-islets without diabetes being present. Herxheimer, studying the cell-islets in the cirrhotic pancreas so often met with in diabetes, found evidences of their new formation from the small ducts, but regarded the whole pancreas as controlling sugar metabolism. He thinks, however, that diabetes may be due to functional changes which may, or may not, be accompanied by visible lesions. He considers that, in man, the islets alone are inadequate for the prevention of diabetes, while, in animals, they seem to be sufficient. This conclusion is not, however, supported by convincing evidence, and is directly controverted, as regards human diabetes, by a case described by S. G. Scott, in which structures having the characters of islands of Langerhans were found as the only recognisable remains of the pancreas in a mass of fibrous tissue associated with malignant disease of the head of the gland, yet there was no trace of sugar in the urine. Pathological changes in the cell-islets without diabetes have been described. Chauffard and Ravant met with swelling and increase in size of the islands without glycosuria in thirteen cases of enteric fever, two of pneumonia, and one of erysipelas. Salisbury Trevor observed similar changes in pneumonia and infective endocarditis.

The balance of available evidence, and the weight of opinion, would seem to be in favour of the view that the islands of Langerhans are specific structures having an internal secretion that is concerned in carbohydrate metabolism. Their connection with diabetes may therefore be assumed, but in view of the conflicting results reported by different observers, many of whom have investigated but a few cases, it is extremely difficult to draw satisfactory conclusions as to the exact part they play, and the nature of the lesions that exist. It is evident that what is needed is a careful correlation, by unprejudiced observers, of the conditions present in a number of patients during life, with the state of the pancreas and cell-islets after death. Statistical studies are here of importance. Sauerbeck in 1902 collected from various sources reports of 176 cases of diabetes in which the condition of the cell-islets had been noted, and found that in 117 (66 per cent.) some abnormality had

been observed. If, however, the purely quantitative changes were excluded as being of too indefinite a character, there remained ninety-eight (62 per cent.) in which qualitative changes had been met with. The most extensive collection of cases yet made by one observer was published by Cecil in 1909. He investigated the pathological anatomy of ninety cases of diabetes, and came to the following conclusions : (1) Anatomical lesions of the pancreas occur in more than seven-eighths of all cases of diabetes mellitus. (2) When lesions are found, the islands of Langerhans constantly show pathological changes (sclerosis, hyaline degeneration, infiltration with leucocytes, and hypertrophy). (3) In some cases (12 out of 90) the lesions are limited to the islands of Langerhans. (4) In sixteen cases associated with hyaline degeneration of the islands of Langerhans. the average duration of the disease was three and a half years ; and in forty-six cases with sclerosis of these bodies the average duration was $3\frac{11}{12}$ years. In six cases associated with an infiltration of leucocytes the average duration was eleven months. (5) Destructive lesions of the islands of Langerhans may be associated with compensatory hypertrophy of other interacinar islands. (6) Peculiar adenoma-like hypertrophy of the islands of Langerhans occurred in a small proportion of cases (7 out of 90), and was associated with adenomata of the thyroid gland in two cases, and of the pituitary body in one. (7) Fifty per cent. of cases of diabetes mellitus, occurring before the age of thirty years, are associated with lesions of the pancreas ; 75 per cent. of all cases of diabetes, in which the pancreas is normal, occur before the age of thirty years ; 97 per cent. of cases, occurring after the age of thirty, are associated with lesions of the pancreas, and 86 per cent. occur in conjunction with chronic interacinar pancreatitis accompanying arterio-sclerosis. (8) Interacinar pancreatitis, which occurs in 73 per cent. of cases, is almost constantly associated with arterio-sclerosis and gangrene of the extremities, which occurs with one-fourth of cases of interacinar pancreatitis, and is referable to the same cause. (9) Chronic interlobular pancreatitis, when associated with diabetes, is accompanied by sclerosis or hyaline degeneration of the islands of Langerhans.

The association of arterio-sclerosis, gout, syphilis, and alcoholism with chronic glycosuria is probably to be explained by the fibrotic, and degenerative, changes which each is capable of setting up in the pancreas. As a rule the diabetes is of a mild type, such as might be expected from a slowly progressing interstitial pancreatitis. The glycosuria consequent on the presence of gall-stones is, in my experience, usually of a similar mild type. The apparently in-

fective origin of some cases of diabetes may be due to the effects produced upon the pancreas by micro-organisms entering the ducts from the duodenum, and the history of digestive disturbances given in many cases suggests that a chronic duodenal catarrh may, in some instances, give rise to conditions favourable to the onset of a pancreatic lesion that eventually causes glycosuria. Hirschfeld has published cases which suggest that pancreatitis, with secondary glycosuria, may be set up through an infection carried by the blood, and he thus explains the sugar sometimes met with in the urine after influenza, tonsilitis, and various infective processes.

A few cases have been reported in which there has been no glycosuria, although the whole of the pancreas appeared to have been destroyed by malignant disease, or inflammatory processes; but none are of recent date, and in most instances the proof of total destruction has rested upon naked eye examination alone.

Even if the connection of diseases of the pancreas, including more or less marked lesions of the islands of Langerhans, with diabetes is granted, a certain number of cases remain in which no marked morbid change could be discovered in that organ by competent observers. Opie met with four in his nineteen cases, Williamson with eight out of twenty-two, Ssobolew with two out of fifteen, and in twenty-three examined by Schmidt there were no pathological changes in the pancreas in eight, and in eight others the alterations were so slight as to be considered secondary to the diabetic condition. Cecil states that in 12 per cent. of the cases he examined no distinctive morbid condition of the pancreas could be discovered, although in approximately half of these he considers that the size of the gland, or the number of islands of Langerhans, was less than normal. It is therefore probable that only a proportion of cases of diabetes, possibly some 75 per cent. at the outside, can be referred to structural alterations in the pancreas. There are some who suggest a higher figure, and a few who have claimed that all cases of diabetes are of pancreatic origin, but while allowing that there may be fine histological and chemical changes, which we are unable to detect by the methods at present available, most authorities are now agreed that there is both clinical, and experimental, evidence, that the pathology of all cases of diabetes is not the same, and that some are to be referred to other causes.

Supra-renals.—Although the experimental evidence in favour of the view that the supra-renals control the metabolism of carbo-

hydrates through their internal secretion is now considerable, the clinical facts pointing to disease of these structures being responsible for diabetes is as yet very meagre, possibly owing to insufficient care having been devoted to their investigation. Burghart described in detail a case of diabetes occurring in a woman of thirty-nine in which the pancreas was normal, and a sarcoma of the left supra-renal capsule was found post-mortem. Three months before death she suddenly developed thirst and polyuria, and her urine was found to contain 8 to 10 per cent. of sugar. Lépine has reported a case of sarcoma of the right supra-renal capsule in a woman of sixty-four, whose urine contained 9 grams of sugar per litre, and who died comatose. There was a history of loss of flesh for two years, and great thirst for one and a half years before death, so that there is a possibility that the diabetes antedated the growth. Tumours of the supra-renals have also been observed by Grawitz in association with diabetes. Ogle has described a case in which small patches of fibrinous material were found in the centre of each adrenal. This case is referred to by Naunyn as one of tuberculosis. Rabe has reported an example of what he considered to be co-existing bronzed diabetes and Addison's disease, in which the supra-renals were totally destroyed by tuberculosis. A case of diabetes mellitus in which the right adrenal was replaced by a small fibrous mass, showing caseous tuberculous changes, and the left was larger than normal has been described by Montgomery. In this instance there was also chronic interstitial pancreatitis. but the islands of Langerhans were said to be normal. The thyroid was thought to be atrophied, and there was arterio-sclerosis. The urine contained from 2·5 per cent. to 3·5 per cent. of sugar, 38 to 40 oz. being passed in the twenty-four hours. It had a specific gravity of 1·024 to 1·028. Acetone and aceto-acetic acid were present. Montgomery came to the conclusion that there was no causal relationship between the primary tuberculosis of the supra-renal and the glycosuria, and that the association of the two conditions was merely a coincidence.

Hyperactivity of the supra-renals has been supposed by Lépine to be the cause of the glycosuria observed to follow prolonged hypertension, Neubauer has detected hyperglycæmia in patients with chronic renal disease and a high tension pulse, and other observers have met with hypertrophy of the chromaffin tissues and hyperadrenalism in such cases. Examples such as these, considered in the light of the experimental work that has been done, are suggestive, but the evidence of the interdependence of the glycosuria and a pathological condition of the supra-renals, and

more especially of growths of these structures, is so slight that an accidental association cannot be excluded.

Garrod points out that malignant growths originating in the adrenal medulla are not uncommon in children, yet, in his experience, sugar is never found in the urine, nor is there a raised pulse tension to suggest an excess of adrenalin in the blood. We must remember, however, when considering the effects produced by disease of the supra-renals that chromaffin cells, resembling those of the medulla of those glands, are found in other situations in connection with the sympathetic nervous system, as strands in the larger nerves and as oval masses about the ganglia, the so-called paraganglia, especially in the carotid gland, and in the lower animals, in the abdomen at the point of division of the aorta (Zuckerkandl's organ), and in the coccygeal glands, &c. Swale Vincent found that an extract of the abdominal chromphile body of the dog has the same powerful effect upon blood pressure as an extract from the medulla of the adrenals, so that it would appear that the other chromaffin tissue of the body has an internal secretion resembling that of the supra-renals. Destruction of the supra-renals by a growth may therefore be compensated by hypertrophy of the remaining chromaffin tissue, while any influence that causes hyperactivity of the chromaffin tissue as a whole will tend to produce glycosuria. The affinity of epinephrin for structures that are, or have been, in intimate connection with sympathetic nerves, and the close relation of the chromaffin tissue to these nerves, offers an explanation of the way in which a very slight increase in the activity of the chromaffin tissue cells may produce results apparently out of all proportion to the intensity of the lesion present. The experiments of Woolley and Newburgh on the results produced by the injection of indol and tyrosin into animals suggest that these, and possibly other lower derivatives of protein decomposition in the intestine, may cause hyper-activity of the adrenals, and hence be a factor in disturbing the pancreas-chromaffin equilibrium, and give rise to glycosuria as a consequence.

It may be assumed that in Addison's disease a condition of hypo-adrenalism exists, and that, if the supra-renals have the function in metabolism that is ascribed to them, not only will glycosuria be unknown, but that tolerance for sugar will be raised. Porges has shown that the blood in this disease contains an abnormally low proportion of sugar, and he, as well as Eppinger, Falta, and Rudinger, state that patients suffering from Addison's disease exhibit an abnormally high tolerance for sugar. In seeming contradiction to these results, however, is the case recorded by

West, in which, although the supra-renals were completely atrophied and the skin was pigmented, there was well-marked glycosuria. Unfortunately no information is furnished as to the condition of the pancreas and other viscera in this case.

In view of the small amount of evidence available, and its somewhat contradictory character, it is impossible to come to a definite conclusion as to the effects of disease of the supra-renal capsules on carbohydrate metabolism, but it seems not unlikely that some cases of persistent dextrosuria are dependent upon hyperfunction of these structures, and possibly also of the remaining chromaffin tissue of the body.

Pituitary Gland.—One of the earliest reported cases in which disease of the pituitary gland was found to be associated with glycosuria was that of Lépine. This patient suffered from acromegaly, and during life passed about 7·1 per cent. of sugar in her urine. After death a large tumour of the pituitary was found. Four years later Marinesco published a similar case. In this instance the acromegaly was known to have antedated the glycosuria by about three years. Cases in which acromegaly has been associated with the presence of sugar in the urine have been reported by Naunyn, Williamson, Löeb, Launois, and Roy, and others. Hansemann found that diabetes was present in twelve out of ninety-seven cases of acromegaly, and Schleisinger described three cases—one with diabetes, one with alimentary glycosuria, and one with transient glycosuria. Borchardt, reviewing the literature in 1908, states that in 176 cases of acromegaly, glycosuria had been observed in sixty-three (35 per cent.), and that there was a lowered tolerance for sugar in eight others. The latter symptoms had apparently been looked for in only ten cases. He points out that in all the cases of pituitary tumour recorded between 1886 and 1908 in which glycosuria was present there were acromegalic symptoms.

As a rule the sequence of the symptoms has left little doubt that the glycosuria was a consequence of the disease of the pituitary giving rise to the acromegaly. Occasionally, however, sugar has been found in the urine before the symptoms of acromegaly appeared, as in a case quoted by Schleisinger. In this instance sugar was detected in the urine at the age of six, but under treatment it disappeared until the symptoms of acromegaly were manifested, when it appeared anew. Subsequently it was found at irregular intervals, and without any apparent relation to the diet. A similar intermittence in the glycosuria has been noted in other cases. Thus Strümpell had under observation for several years a

case in which thirst and polyuria were prominent symptoms and the urine contained as much as 100 to 120 grams of sugar in the twenty-four hours, yet during two separate periods, with an interval of four years between them, the sugar completely disappeared in spite of a diet containing an abundance of carbohydrate. In a few instances the glycosuria, after persisting for years, has permanently disappeared. Such cases are referred to by Borchardt and Pineles.

Goetsch, Cushing, and Jacobson investigated a series of twenty cases of acromegaly, or gigantism, and failed to discover sugar in the urine of any one of them. They found, on the contrary, that there was a marked increase in the tolerance for carbohydrates. The results of the animal experiments carried out by these observers, to which reference has already been made, appear to offer an explanation of the difference between their findings and those which the experience of previous authors would lead one to expect, for they suggest that in the early stages of the disease there is hyperfunction, not only of the anterior lobe, which leads to the skeletal changes, but also of the posterior lobe, leading to increased carbohydrate tolerance. It may also be mentioned here that, according to Goetsch, Cushing, and Jacobson, primary hypofunction of the posterior lobe may result from direct pressure by interpeduncular tumours, by growths originating in the lobe itself, and by the distant effects of a tumour causing an obstructive hydrocephalus, and thus damming back the fluid medium carrying the posterior lobe secretion. In such cases the same symptoms are seen as in the later stages of acromegaly. There is a high tolerance for sugar, marked adiposity, due to a lowered consumption of sugar, and a subnormal temperature, perhaps arising from deficient oxidation. The administration of posterior lobe extract lowers the carbohydrate tolerance, and by giving sugar in the amounts usually just sufficient to cause glycosuria in normal individuals (about 2 grams of dextrose or 1·4 grams of levulose per kilogram), and administering posterior lobe in increasing doses at the same time until glycosuria is produced, it is possible to determine the proper therapeutic dose of the gland for each case. Cushing and his associates are also inclined to think that the transient glycosuria seen in cases of fracture of the base of the skull is due to temporary stimulation of the hypophysis, and that over-activity of the gland also explains the polyuria that occurs after such accidents. The earlier hyperactivity of the posterior lobe may later give way to a condition of hypo-activity with increased carbohydrate tolerance, as in a case described by these authors.

The quantity of sugar excreted in acromegaly and gigantism is often very large, but it frequently varies considerably. In Achard and Loeper's case 380 grams were passed in the day, Lancereaux's case passed 6 to 8 litres of urine containing 180 to 240 grams of sugar in the twenty-four hours, that of Perwuschin and Foworski 3 to 8 litres, containing 30 grams of sugar per litre, while in Ravaut's case from 15 to 20 litres, containing 500 to 1500 grams of sugar, were excreted daily during several months. As a rule there are no symptoms of any secondary disturbance of metabolism such as are seen in severe cases of diabetes. Ravaut and Prau with Proescher have met with acetonemia, but there appears to be only one recorded case, that of Stadelmann, which died of diabetic coma. Herxheimer has reported a case in which there was lipæmia.

In some cases of acromegaly with glycosuria structural lesions of the pancreas have been described, both of the gland as a whole and of the islands of Langerhans. Dallemange, Hansemann, Pineles, Norris, and Cecil have observed sclerosis and other pathological changes. The alterations in the pancreas appear, however, to have been comparatively slight, and Borchardt states that in most cases the gland has been found normal. That disease of the pancreas can exist in acromegaly without there being any glycosuria, is shown by Stadelmann's case, in which partial sclerosis was found after death. On the other hand, in the fatal case quoted by the same author in which there was severe diabetes and death occurred from diabetic coma, the pancreas was stated to be quite healthy. Cecil considers that the glycosuria in acromegaly may be referred to lesions of the islands of Langerhans, chiefly sclerosis and hyaline degeneration, with adenoma-like hypertrophy. Most authorities hold, however, that the interference with the internal secretion of the hypophysis is the primary cause of the glycosuria, and that any lesions of the pancreas that may be present are probably secondary.

An increase in the volume of the thyroid has been noticed in some cases, as, for example, those of Ferrand, Lancereaux, and Henrot. In the two latter there were also the symptoms of exophthalmic goitre.

The Thyroid.—It has been already pointed out that the tolerance for sugar of patients suffering from hypothyroidism (myxœdema) is increased, while with hyperthyroidism (exophthalmic goitre) it is lowered, so that alimentary glycosuria can be readily produced. A number of cases have also been reported

in which intermittent, or persistent, glycosuria has been associated with Grave's disease. Dumontpellier in 1867 appears to have been the first to describe such a case. In his patient the urine contained 6 per cent. of sugar. A few years later Lauder Brunton reported a similar case, and in the following year Wilks gave an account of a patient who passed 300 grams of sugar a day. In 1878 Hartmann reported two cases of exophthalmic goitre with glycosuria, and O'Neill described a similar case. Since then a considerable number have been recorded by other authors. In many of these there is no evidence to show which condition preceded the other ; but as up to the present there has been only one recorded case, that described by Grübe, in which glycosuria was the first symptom, and there is no evidence to suggest that diabetes can give rise to exophthalmic goitre, it is not unfair to assume that the condition of the thyroid is in some way responsible for the diabetes, especially as this is in accordance with the results of experimental work. It may also be noted that in a few cases exophthalmic goitre and diabetes have occurred in different members of the same family. Such have been reported by Mautry and Schmey, and Garrod states that he has had under his care a young woman suffering from the severest form of diabetes whose mother had Graves' disease.

It would thus seem that in exophthalmic goitre all the three degrees of disturbed carbohydrate metabolism may exist. In some glycosuria only results when sugar is taken—that is to say, it is of the alimentary type. Occasionally only very small doses, 20 to 30 grams, are needed to cause sugar to appear in the urine, while in others as much as 100 grams are required. In a few instances the test dose may cause a glycosuria that persists for several days, suggesting that the patient is on the verge of persistent spontaneous glycosuria. With the second type of case, into which the last mentioned merges, sugar is present in the urine at intervals, sometimes for a day, at others for several days, probably owing to the patient's ordinary diet exceeding his tolerance at these times. In the third class of case glycosuria is persistently present, although it may be controlled by diet as in other forms of diabetes.

Soon after the introduction of thyroid preparations into clinical medicine it was noticed by Dale James, and Béclère, that their continued use might result in a reduction of tolerance for carbohydrates and the spontaneous appearance of sugar in the urine, also that on the drug being discontinued the glycosuria promptly disappeared. These results are most readily induced in patients

whose thryoid secretion is normal. Thus Garrod reports a case in which a thyroid preparation was given to reduce obesity with the result that sugar, to the extent of 4 per cent., appeared in the urine at the end of a week, when only 4 grams had been taken. In myxœdema a similar effect may, however, follow the continued use of the drug. Thus Ewald has reported a case in which glycosuria occurred on three successive occasions after the administration of thyroid extract in very moderate doses, such as 5 grains a day, as much as 5 to 6 per cent. of sugar appearing in the urine. In the intervals the urine was free from sugar. Garrod states that an examination of the urines of eleven myxœdematous patients, who had been under treatment with thyroid extract in St. Bartholomew's Hospital, showed that four contained sugar, and that the glycos-uria quickly disappeared when the treatment was stopped. It has been thought that in some instances the administration of thyroid has given rise to actual diabetes. Von Noorden suggests that in such cases a pre-existent diabetic tendency has been awakened into activity by the drug. Müller has described a case in which rapidly fatal diabetes occurred in a patient with exoph-thalmic goitre who was treated with thyroid extract.

It must be pointed out that in many cases of exophthalmic goitre no diminution of tolerance for sugar can be detected, while, on the other hand, some patients suffering from myxœdema, or at least from thyroid insufficiency, have passed sugar in their urine, apart from any treatment with thyroid extract. A few of the latter would seem to be undoubted examples of hypothyroidism, where glycosuria would not be expected, but in others there is room for doubt as to the correctness of the diagnosis. Such seeming ex-ceptions to the generally observed effects of alterations in the functional activity of the thyroid are possibly explained by the interaction of other glands, and only serve to emphasise the diffi-culty that surrounds the study of organs that are so intimately correlated. Some observers, notably Opie and Cecil, hold that the glycosuria of Grave's disease is due to disease of the pancreas, but although lesions of the gland have been found in a few fatal cases, the fact that thyroid medication may cause sugar to appear in the urine can only be reconciled with such a view by supposing that hyperthyroidism produces a lesion of the pancreas.

The glycosuria of pregnancy is ascribed by Reichenstein, Cushing, and others to the changes that occur in the ductless glands, and more particularly in the thyroid and pituitary body. Enlarge-ment of the thyroid is a well-recognised event during pregnancy, and changes in the pituitary have been shown to be common by

Erdheim and Stümme. The case recorded by Marek tends to support the view that changes in the pituitary gland may be in part responsible for the glycosuria in such cases.

The patient, a primipara, aged twenty-six, developed the characteristic symptoms of acromegaly during the eighth month of pregnancy. Her hands and feet enlarged, her skin, nose, and lips were thickened, and enlargement of the lower jaw prevented her teeth from meeting. She became very somnolent. Sugar was present in her urine. After delivery the glycosuria ceased in about two months, and by that time all signs of acromegaly had also disappeared. It can hardly be doubted that the glycosuria and attendant symptoms were alike due to hyperpituitarism developed in association with the pregnancy.

It will be convenient to refer here to the glycosuria that has been observed in association with diseases of the *female generative organs*. Imlach described a case in which removal of the uterine appendages, on account of pyosalpinx with ovarian adhesions, was followed within a week by cessation of glycosuria which had been detected five months before the operation, and which was accompanied by thirst and polyuria. In cases recorded by Croom, Beyea, and Henkel, removal of ovarian tumours, or uterine myomata, has in like manner been followed in the course of a few months, and apart from restriction of diet, by disappearance of sugar from the urine. In Beyea's case the glucose amounted to as much as 7 per cent. It will be noticed that in most cases the cessation of the glycosuria has been much more gradual than after childbirth.

Nervous System.—There can be no doubt that nervous disturbances enter into the pathology of many cases of diabetes, for not only are there numerous well-authenticated cases where severe or fatal diabetes has developed as the result of a severe mental shock or an injury to the head, but the harmful effect of worry, overwork, and shock on an established glycosuria are well known.

In such cases it is tempting to suppose that while the primary effect of the emotional stress is on the nervous system, secondary changes are produced in the glands with an internal secretion, and that it is to a disturbance of their functions that the excessive output of sugar is directly due. It is, however, difficult to estimate the exact part that functional and emotional affections play, for they give rise to no obvious lesion, but it is otherwise with the persistent glycosuria that is met with as a result of gross morbid changes in the brain, spinal cord, and nerves.

An analysis of the post-mortem records of the Allgemeine Krankenhaus at Vienna by Seegen showed that in only eleven out of

122 cases (9 per cent.) of diabetes was there any marked change in
the nervous system, and that in many of these it was doubtful
whether the lesion could be regarded as the cause of the glycosuria.

In some instances a disease involving the fourth ventricle has
been found. The earliest recorded case of this description appears
to be that reported by Levrat-Perrotton, in 1859, in which a
colloid tumour of the choroid plexus was present. Five years
later v. Recklinghausen published a similar case. Subsequently
other observers reported cases in which glycosuria had been found
in association with disease of the fourth ventricle, including soften-
ing (Richardson, Luys), sclerosis (Frerichs, and others), hæmorrhage
(Frerichs, &c.), cysticerus (Michael). A cystic sarcoma of the right
half of the medulla, with glycosuria, was reported by Dompeling, a
tumour of each pyramid close to the pons by Frerichs, and tubercle
of the medulla by De Jonge. Lesions of the spinal cord, including
tumours compressing the cord in the cervical region (Smith),
hæmorrhage and softening in the cervical and upper dorsal region
(Silver and Irvine), have also been met with in cases of diabetes.
Changes in the cerebral hemispheres, including a tumour of the
right temporal lobe and pachymeningitis, have been reported by
Frerichs, and lesions of the cerebellum by the same observer, and
by Mosler. Tumours at the base of the brain, and arterial changes
with softening, have been described by Richardson, Grossman,
and others. Bernardt collected reports of 485 cases of brain
tumour, and found that sugar was present in the urines of five—
one being a tumour of the medulla, two of the hypophysis, one of
the cerebellum, and one of the cerebral hemispheres. Occasionally
glycosuria has been met with in association with disseminated
sclerosis, locomotor ataxia, chronic anterior poliomyelitis, and
other well-defined diseases of the nervous system, such as menin-
gococcal meningitis (Mannkopf, Adler, and others), tuberculous
meningitis (Löeb, Still, Aldren Turner, Grainger-Stewart, and
Garrod), &c. In most of these glycosuria is a rare complication,
but according to Garrod it is met with in 30 per cent. of all cases
of tuberculous meningitis.

The microscopical changes met with in the central nervous
system in diabetes consist of swelling of the myelin, vacuolar
degeneration of both the grey and white matter, and an increase in
the neuroglia ; but these, like the similar changes seen in perni-
cious anæmia, tubercle, and cancer are more probably the result
of the action of a toxine than the cause of the glycosuria.

In a few cases tumours pressing on the vagus nerve have been
met with (Dulen). Lesions of the cœliac plexus have been de-

scribed by Klebs and Munk. A thickening of the connective tissue
in the neighbourhood of the semi-lunar ganglion was met with
by Hale White in several cases. Cazzani has described lesions of
the sympathetic nerves, particularly in the neighbourhood of the
cœliac plexus.

Traumatism, particularly of the head and spinal cord, has
been noticed to be followed by persistent glycosuria in some cases,
but more frequently the condition is transitory. In 145 cases
investigated by Jodry the head was involved in seventy-two
(50 per cent.), the spinal cord in twenty-seven (20 per cent.), the
abdomen in twelve (8 per cent.), in 5 per cent. the patient had fallen
on his feet, and in 17 per cent. the site of the traumatism was not
clearly indicated. According to Lépine, the glycosuria manifests
itself the day after the injury in a third of the cases, and in the
majority during the first week. In a few cases, about 20 per cent., it
may be delayed, however, for two or three weeks, or even longer.

As a rule the quantity of sugar passed is small, not usually
more than 8 to 10 grams per litre. According to Garrod, the sugar
in tuberculous meningitis rarely exceeds 1 per cent., usually being
about 0·3 per cent., and appears during the last week of life,
generally during the last two days.

Although in a small proportion of cases of persistent glycosuria
a definite lesion of the nervous system is found, it may be con-
cluded that in the majority it is either normal, or only presents
slight and unimportant alterations. It is very generally assumed
that in those instances where there is some gross cerebral change
the glycosuria results from implication of the diabetic centre.
This may be the explanation in a very few, but it is probable that
in most cases other causes are the active agents in its production.
The relation of the nervous system to the ductless glands that has
now been worked out, suggests that it is through disturbances in
the functions of these organs, either directly, as in the case of the
pituitary, or indirectly through nervous influences, as in the case
of the supra-renals, that the hyperglycæmia and consequently
glycosuria result.

A special form of neurogenic glycosuria is recognised by
v. Noorden, which he considers is comparatively harmless and is
controlled by suitable restriction of the diet. If neglected or
mismanaged it may, however, be converted into a typical diabetes.
A case of this description has been recently published by him in
detail.

The patient, a man of forty, had an inherited neuropathic taint,
but the glycosuria did not develop until the age of thirty-six, following

Q

a period of great anxiety connected with the massacres in Russia. Severe insomnia, loss of appetite and weight, and obstinate constipation, were accompanied by 1·5 per cent. sugar in the urine, persisting even on anti-diabetic diet. Sleepless nights were followed by exacerbation of the glycosuria. Under slight restriction of carbohydrates, with one day a week in which no carbohydrates were taken, the patient lost his glycosuria. He kept well on this diet for a year. Then another physician found the urine free from sugar on two analyses, and advised the patient that anti-diabetic restrictions were no longer necessary, so he commenced to eat at will. It was not long before he had pains in the calves of the legs, he soon began to lose weight, and 5·6 per cent. sugar was found in the urine. Attempts to reduce the carbohydrates brought on acetonuria, and the man is now a confirmed diabetic.

Liver.—From the importance of the liver as a storehouse of carbohydrates, it might be expected that striking lesions of that organ would be found in a considerable number of cases of diabetes. Glycosuria is, however, very rare, even when it is apparent that the functions of the gland must be seriously interfered with. Clinical and pathological experience show that extensive destruction of the parenchyma may occur in cancer, cirrhosis, phosphorus poisoning, and other diseases without even a trace of sugar appearing in the urine. Frerichs, who investigated the liver in fifty-five cases of diabetes, came to the conclusion that it is generally normal in volume, sometimes small, and very rarely enlarged. Saundby, on the other hand, states that it is generally enlarged, weighing 68 to 80 ounces. The latter opinion agrees with the more recent observations of Rössle, who considers that a diagnosis of diabetes can be made in the post-mortem room with more certainty from the appearance of the liver than from the condition of the pancreas. He states that, while the normal liver constitutes from 2·3 to 2·75 per cent. of the body-weight, in diabetes it represents from 2·9 to 4·1 per cent., averaging 3·54 per cent. Beside the enlargement, it is usually of a rosy colour, and the parenchyma is transparent, or homogeneous, in appearance. Microscopically the diabetic liver, according to this observer, shows interstitial changes, including very constantly fatty degeneration of the stellar cells of Kupffer, and homogeneous refractile bands along the capillaries, which he believes to be specific and characteristic of diabetes. It is generally considered, however, that there are no constant macroscopical or microscopical changes to be found in the liver. Beside enlargement, hyperæmia, and fatty degeneration, which are probably of a secondary nature, and are possibly the result of hyperfunction of the gland, cirrhosis is the most common lesion met with. Claude

Bernard in his lectures on diabetes described a case of cirrhosis of the liver, occurring in an old alcoholic subject, who in the early months of 1873 passed 6 litres of urine a day, containing 29 grams of sugar per litre. Later, as his condition became more serious, and the cirrhosis advanced, the sugar began to diminish, until only a trace could be found. The glycosuria was attributed by Bernard to excitation of the glycogenic function of the liver by the disease in its early stages, and the disappearance of the sugar to its extinction in the later phases. Other observers have also reported the association of diabetes with cirrhosis of the liver, and in some instances the glycosuria has been found to diminish or disappear with the onset of cachexia. Among 128 cases of diabetes observed in hospital by Naunyn seven, and of 158 in his private practice twenty-two, were found to have cirrhosis of the liver. The association appears to be rather accidental than causal, for Frerichs has recorded cases of cirrhosis in which post-mortem examination showed almost complete degeneration of the liver parenchyma, and yet there was no glycosuria during life, and even a large quantity of sugar taken by the mouth did not produce it. An explanation of these seemingly opposing results was offered by this observer, who pointed out that chronic inflammation of the liver and pancreas are very commonly found together, and that the glycosuria seen in cases with cirrhosis of the liver is probably dependent upon an associated pancreatic lesion. Chvostek, Hansemann, Dieckhoff, Oser, Opie, and many others, have since confirmed the frequency with which chronic inflammatory changes are met with in the pancreas and liver at the same time.

The researches of Lefas are of particular interest, as he investigated the alteration in the pancreas accompanying different varieties of cirrhosis. With atrophic (Lænnec's) cirrhosis he found that the weight of the pancreas was often increased, and that there was a uniformly intralobular overgrowth of connective tissue, poor in cells, penetrating the parenchyma and isolating groups of acini. With hypertrophic biliary (Hanot's) cirrhosis the pancreas was not enlarged, but the interlobular tissue was increased in amount and density. The size and consistency of the pancreas did not, however, appear to be related directly to the condition in the liver. As a rule the liver was more seriously affected, but in some cases the disease of the pancreas was in a more advanced stage. In every case, however, the fibrous tissue in the pancreas was fully formed and poor in nuclei, even when the newly formed fibrous tissue in the liver was of a semi-adult type. It is therefore concluded that the cirrhosis of the liver and of the pancreas are due

to the same etiological factors, but that the condition of the pancreas is independent of, and not secondary to, the lesion in the liver. Hirschfeld considers that, as a rule, the liver and the pancreas are simultaneously affected, but the liver affection generally retrogresses, although traces of the process can be discovered by the pathologists. If the liver is in any way deteriorated, as from the action of alcohol or malaria, the process in the liver predominates, and although cirrhosis may develop in the pancreas, yet no diabetes results. He thinks that the portal vein is very rarely the route by which the influences responsible for the changes in the liver and pancreas arrive. Hirschfeld cites some cases described by Tsuchiya in which cirrhosis in the liver was the direct result of invasion of the gland through the portal vein by helminths, the *Schistosoma japonica*, where the pancreas was intact, and accepts this, as confirming his view that when the infectious agent arrives by the portal route the liver alone is affected, but when it comes by way of the blood both liver and pancreas suffer.

A striking illustration of the dependence of chronic lesions of the pancreas and liver upon the same etiological factor is furnished by the condition known as hæmochromatosis, described by v. Recklinghausen in 1889. In this a reddish-yellow iron-containing pigment is deposited in the epithelial cells of the liver, pancreas, and other glands, while a yellow iron-free pigment is found in the muscles of the gastro-intestinal tract, blood-vessels, &c. Associated with the pigmentary deposit there are cirrhosis of the liver, chronic interstitial pancreatitis, and histological lesions of the stomach, intestine, heart, spleen, kidneys, &c.

Closely related to hæmochromatosis is the condition described by Hanot and Chauffard in 1882 as "*Bronzed Diabetes.*" In this there is a rapidly fatal form of diabetes with cirrhosis of the liver and pancreas, associated with pigmentation of the internal organs and skin. The bronzing of the skin is usually general and uniform, but is not accompanied by pigmentation of the buccal mucous membrane, as in Addison's disease. Although found in the majority of cases it is not a constant symptom, being absent in some 15 to 16 per cent. The disease is almost exclusively met with in males, and usually between the ages of thirty and sixty. Alcohol is stated by some to play an important part in the etiology of many cases. Hanot and Chauffard believed that the diabetes was the primary factor in the disease, the changes in the liver and pancreas being the result of alterations in the blood and the accompanying endarteritis. Marie, Acard, and Dutourier, Jeanselme, and Ancshultz came to the conclusion that the tissue changes are

the consequence of the deposit of pigment in them, and that the pigment arises from the dissolution of hæmoglobin from some unknown cause. According to this view the diabetes is a phenomenon secondary to changes in the pancreas. Opie is of opinion that hæmochromatosis is a distinct morbid entity associated with chronic interstitial inflammation, notably of the pancreas and liver. He considers that diabetes only ensues when the pancreatitis has reached a certain grade of intensity, and is usually the terminal event. He found that the pancreatic inflammation is of the interacinar type, and that the islands of Langerhans are implicated in the lesion, their destruction being, in his opinion, responsible for the glycosuria. Cecil agrees with Opie's conclusions, and states that the diabetes associated with hæmochromatosis is referable to pigmentation and destruction of the islands of Langerhans. Margin found that in a fatal case of bronzed diabetes that he examined, some of the islands of Langerhans were preserved, but that their cells were crowded with pigment. Potter and Milne, from what they have found in the literature, and from a study of a case under their own care, came to the conclusion that cirrhosis of the liver is the primary condition. They consider that pancreatic involvement with diabetes is a sequence, or coincident event, to this ; that the hæmochromatosis, always present in slight degree in liver cirrhosis, is in some cases very excessive and causes a general pigmentation which eventually also involves the skin ; and finally, that the whole process is not definite symptom-complex, but a chain of circumstances which rarely seems to be complicated. In a review of the literature up to 1910 Bernoulli found forty-one detailed reports of cases of so-called bronzed diabetes, and states that in seven there was no tendency to glycosuria.

Gastro-intestinal Tract.—The stomach in diabetes may show evidence of gastritis, and in a few cases atrophy of the mucosa has been described. The most common morbid condition is hyperplasia of the gastric and intestinal mucous membrane. Hédon states that animals, which have survived for some time after extirpation of the pancreas, show a thickening of the walls of the stomach and intestine, with hypertrophy of the mucosa, and according to Martinotti and Boccardi there is an abnormal development of the glands of Lieberkühn. It has consequently been suggested that the hypertrophy met with in human diabetes is secondary to disease of the pancreas. Others, however, are inclined to regard the changes in the bowel as primary, and to consider that there is a glycosuria of intestinal origin.

Reale and Renzi, in 1890, stated that after extirpating the duodenum in dogs glycosuria results, the animal passing 15 grams or so of sugar in its urine in the following twenty-four hours. Subsequently this question was taken up by Pflüger, who, as we have seen, came to the conclusion that there exists in the wall of the duodenum an " anti-diabetic centre " which controls the pancreas. Herlitzka found that glycosuria could be produced in frogs by injecting nicotine into the duodenum, owing, he thought, to a paralysis of the sympathetic nerves. Pflüger's conclusions were, however, denied by Ehrmann, Rosenbaum, Minkowski, and others, and Lépine failed to induce glycosuria in dogs by injections of nicotine. Eichler and Silbergleit, in consequence of the description by Zak of two cases of corrosive poisoning, one by an alkali and the other with a mineral acid in which glycosuria occurred, made experiments upon dogs, and found that severe corrosion or scorching of the duodenum was followed by the appearance of sugar in the urine. The same result followed similar treatment of other portions of the intestine, however, and they came to the conclusion that the glycosuria was due to an emptying of the glycogen reservoirs, such as occurs after other violent insults to the organism.

Another explanation of the association of glycosuria with gastrointestinal diseases is that an extension of an infective process to the pancreas takes place. My own experience suggests that in some instances diabetes may result from such a cause. The following case seems to be an example.

In May 1904 I was asked to examine the urine of a girl of twenty-three, who was complaining of indefinite abdominal symptoms, with loss of appetite and general ill-health. I found a positive " pancreatic reaction," a considerable excess of indican, and a rather high proportion of ethereal sulphates, and consequently diagnosed " catarrhal pancreatitis, probably secondary to a gastro-intestinal catarrh." It was stated, however, that there was no evidence clinically of disease of the pancreas, and as the surgeon in charge of the case considered that the symptoms were due to appendicitis, he operated for that disease and found a subacutely inflamed appendix. In June 1906 I was asked to see the patient, and found that she was much wasted and was passing 5 per cent. of sugar in her urine. She still gave a well-marked " pancreatic reaction," and I diagnosed " pancreatic diabetes." The patient was put on a restricted diet and treated for that condition, but the disease was now too far advanced to hope for any very great improvement and she died a year later.

In this case the diabetes was apparently due to disease of the pancreas secondary to an intestinal catarrh. The pancreatitis certainly existed for two years, and probably for more, before the

destruction of glandular tissue had advanced sufficiently far to interfere seriously with carbohydrate metabolism.

The experiments of Woolley and Newburgh on the effects produced by the injection of the lower derivatives of protein decomposition on the supra-renals, offers yet another possible explanation of the glycosuria that has been found in association with intestinal disturbances, and would also account for the improvement that follows measures taken to control intestinal putrefaction and catarrh in some cases. It is tempting to think that this may have been what happened in Richartz's case.

The patient was a man aged sixty, whose father and brother had died of diabetes. He stated that he himself had excreted sugar eight years previously when suffering from diarrhœa. With a fresh attack of diarrhœa, which lasted for some weeks, 1·4 per cent. of sugar was found in the urine. The motions were of a light yellow colour and foul smelling. Under treatment with milk diet, peptone, castor-oil, and tanalbin the stools became less frequent, improved in character, and ultimately became normal. At the same time the sugar diminished, and completely disappeared in about a fortnight. Afterward it was found that starchy foods did not give rise to glycosuria, but that 30 grams of dextrose still caused sugar to appear in the urine.

Hürter has published a remarkable case of glycosuria in a child, associated with gastro-intestinal symptoms.

The patient was a girl of ten, whose parents were healthy, but some of whose relations had suffered from diabetes. In July 1908 she had an attack of intestinal catarrh, accompanied by urticaria, which had also affected two other members of her family at the same time. There were several recurrences of the symptoms subsequently. Four weeks before the child came under observation she had suffered from vomiting, after a slight indiscretion in diet, and from that day there had been excessive hunger and thirst. She had lost flesh, her urine was often abundant, and sugar was detected in the third week of the illness. Physical examination revealed nothing abnormal in the abdomen or nervous system, but the urine contained 9 per cent. of sugar, and gave a slight reaction with perchloride of iron. As the sudden onset suggested an affection of the pancreas, Schmidt's test diet was given and his "silk-bag" test applied, but no evidence of pancreatic disease was obtained. The patient was strictly dieted, and the output of sugar fell from 210 to 5 grams in the twenty-four hours. In about a week, and after several days of vegetable diet, the glycosuria disappeared entirely. On a single occasion some ten days later a reaction for sugar was obtained. Seven months later it was found that the patient had gained 6 kilos, and that all the symptoms had disappeared. A test diet containing 420 grams of white bread caused no glycosuria, and 50 grams of glucose was also without any effect.

Since then the patient has resumed an ordinary diet, and up to 1910 the urine had remained sugar-free.

This case is particularly interesting because the excretion of sugar was large, yet a perfect recovery apparently took place ; moreover, the patient was a child with whom the prognosis is usually grave.

Cases of intestinal disease with glycosuria have also been reported by other observers, including Schmidt, Funck, and Garrod. The latter points out that some of the most convincing recoveries from diabetes have been associated with gastro-intestinal disturbances, and that this field of inquiry promises to yield fruitful results, and holds out hope of therapeutic advances.

Kidneys.—Post-mortem examination shows that the kidneys are frequently abnormal in those who have suffered from persistent glycosuria during life. Seegen states that at the Vienna Pathological Institute from 1870 to 1892 disease of the kidneys was found in forty out of ninety-two cases. The most frequent change was congestion and enlargement, but small contracted kidneys were not uncommon, occurring chiefly in gouty patients. The most striking and characteristic change in the kidneys in diabetes is the glycogenic degeneration of the epithelium of Henle's tubes, described by Ehrlich and Frerichs. Microscopically the cells are seen to be large and clear. On treating fresh sections with iodine solution the protoplasm stains yellow, and is seen to contain deep brown masses of glycogen. Preparations fixed and hardened in the ordinary way for embedding in paraffin, only show clear round spaces, resembling those left by fat, from which the glycogen has been dissolved out, but the margins, for some unknown reason, tend to take on basic stains. A similar deposit of glycogen has been noticed in depancreatised dogs. The presence of glycogen in this abnormal situation is remarkable in view of its relative deficiency in the more usual situations, and it has been suggested that it is due to the absorption of sugar from the urine. The hyaline degeneration described by Armanni is believed to be a later stage at which the glycogen has disappeared. Diffuse nephritis with fatty degeneration is also met with in some cases.

The onset of inflammatory changes in the kidneys is often associated with a diminution in the amount of sugar passed, and, according to Stocvis, it may completely disappear in cases where there is granular atrophy when the renal changes have reached a certain stage. Even when albuminuria and other evidences of severe kidney mischief are absent, vascular changes sufficient to render the kidneys more impermeable to sugar may be set up by

intercurrent infectious diseases, according to Barrenscheen, thus accounting for the diminished glycosuria frequently met with in such cases.

Other Morbid Changes.—Advanced fatty degeneration of the muscles is a characteristic feature of long-standing cases of diabetes. The heart is usually affected, and the myocardium is pale and soft. Rarely there is hypertrophy of the heart. The spleen is usually small, pale, and soft, but may be enlarged and congested. Croupous pneumonia and broncho-pneumonia, chronic interstitial pneumonia, and tuberculosis, terminating in gangrene, are often found. The lungs may soften (malacia) and mix with the gastric secretions after death, forming the so-called " pneumo-malacia acida."

Theories of Diabetes.—Theoretically glycosuria may be supposed to occur under three conceivable conditions—(1) if the kidneys become abnormally permeable to sugar and allow its escape into the urine; (2) if the sugar in the blood exists in an abnormally loose combination; (3) if the blood from any cause is unusually rich in sugar.

1. It was at one time supposed, and not unnaturally, that diabetes is referable to some disease of the kidneys, which permits the escape of sugar from the blood into the urine, in much the same way that lesions of these organs allow the escape of albumen, but it is now universally agreed that this explanation is not true, and that the morbid changes met with in fatal cases are secondary effects and are not the cause of the glycosuria.

In 1896 Klemperer suggested that there probably exists a variety of glycosuria, which is akin to that caused by the administration of phloridzin, and depends upon a failure on the part of the kidneys to retain the sugar normally present in the blood. This view was accepted by Naunyn, Lüthje, and a few other authors, but most authorities denied that sugar might be present in the urine as the result of such a renal insufficiency. "*Renal diabetes*" is said to be characterised by a slight, but persistent, glycosuria, on which variations in the diet have little or no effect, and by a diminution in the quantity of sugar in the blood. Cases of this description have been carefully investigated and described by Bönniger, Siebke, Weiland, Tachau, and Garrod, and there appears to be little doubt that, though rare, they are a distinct pathological and clinical entity depending upon a functional disorder of the kidneys, possibly arising from the presence of some toxine in the blood which acts in a similar way to phloridzin.

Garrod's case may be quoted as an example.

The patient was a lady between thirty and forty years of age. Her urine was found to contain sugar in 1907 and 1908, the daily output being about 1 gram, but at no time did she exhibit any symptoms of diabetes. She was placed on a restricted diet, but did not bear it well, and the excretion of sugar was, if anything, greater than when she was taking ordinary food. In 1909 Garrod made a series of analyses of her urine and found a total excretion of sugar for the twenty-four hours which varied from 2·0 to 5·3 grams. Restrictions of diet on the one hand, and the administration of 10 grams of glucose on the other, did not obviously affect the glycosuria. Dextrose was definitely proved to be present, but although a great discrepancy between the optical activity and reducing power of the urine was observed, levulose could not be detected. Weiland noticed a similar discrepancy in one of his cases. Unfortunately no estimations of the sugar-content of the blood were made. Analysis of the urine two years and four months later, the patient meanwhile having only avoided sugar and an excess of starchy food, showed no aggravation of the glycosuria.

In Bönniger's case an estimation of the sugar-content of the blood was made.

The patient, a man aged thirty-seven, had for years excreted small quantities of sugar, some 0·2 per cent., in urine with a specific gravity of 1·020. The glycosuria was first detected in the course of an examination for life-insurance, and was proved to be due to dextrose. It varied little in amount, and was not influenced by diet. The administration of 100 grams of dextrose in no way affected it. The blood serum was found to contain 0·097 per cent. of sugar, as compared with the normal of about 0·1 per cent. or rather less.

In Weiland's three cases the average quantity of sugar in the twenty-four hours urine was 7 to 10 grams in the first case, 3 grams in the second, and 10 to 20 grams in the third. In none of them was there any hyperglycæmia. The diagnosis in cases such as these must lie between ordinary mild diabetes, and glycosuria the result of abnormal permeability of the kidneys for sugar. But even if the metabolic findings seem to indicate the latter it is well to adopt an expectant attitude, for careful observation of the case through years is the only means of finally deciding the question.

2. Lowei conceived the idea that the blood sugar is normally in a loose combination with colloid, so that it cannot pass through the glomeruli of the kidneys. If for any reason this combination is not formed, is not broken up, or more sugar is poured into the blood than can be combined with the available colloid, sugar passes through the kidneys and appears in the urine. Stiles and

Lusk have suggested that the colloid sugar exists in two forms, a-colloid and β-colloid dextrose, and that although both can be utilised by the tissues, the latter is more readily attacked, thus accounting for the difference in the dextrose to nitrogen ratio observed in cases of various degrees of severity. These suggestions are, however, highly speculative.

3. There can be no doubt that in the vast majority of cases of glycosuria the presence of dextrose in the urine is due to an excess of sugar in the blood. The limit for normal individuals is somewhat variable, but rarely exceeds one, or at most two, parts per thousand; in diabetes three or four parts per thousand are common, and occasionally cases are met with in which as much as seven or eight parts per thousand exist. Bernard, Pavy, and most observers are agreed that dextrosuria is invariably the result of hyperglycæmia, but Seegen and others, while admitting that this explanation holds good in the majority of cases, believe that in a few of the milder forms of glycosuria the blood sugar scarcely, if at all, exceeds the normal limit. This difference of opinion depends probably in part upon variations in the method employed for determining the sugar, some of which give the glucose content alone, while others also take into account the whole or part of the colloidal sugar. Difficulties also arise from the normal variations met with in different persons, and even in the same patient at different times, and from the fact that only a slight increase of the sugar-content of the blood is followed by its appearance in the urine. Seegen states that glycosuria may occur when the quantity of sugar in the blood is less than 0·2 per cent., an amount no greater than is normally met with in many individuals, although it may possibly be an excessive proportion for some. According to Pavy, the intensity of the glycosuria is directly proportional to the hyperglycæmia; but this is not the experience of Seegen, Naunyn, v. Noorden, and others, who have found that the percentage of sugar in the blood may be only slightly above the normal, in spite of there being marked glycosuria in some cases, while in others there is only a small amount of sugar in the urine, but a high percentage in the blood. The former are probably due to the causes already mentioned, while the latter may possibly be referred to changes in the kidneys, consequent on the persistent glycosuria, which interfere with the elimination of sugar. The fact that hyperglycæmia is the usual cause of glycosuria may now be taken as firmly established.

The question that next arises is, What is the cause of the hyperglycæmia? Numerous explanations have been given, varying

according to the prevailing view as to the primary pathological lesions present in diabetes, but most of these are now only of historical interest. After Claude Bernard propounded the doctrine that the liver is the great storehouse of carbohydrate for the body, and that the sugar of the blood is derived from the glycogen that it contains through the action of an amylolytic ferment, it was concluded that the hyperglycæmia met with in diabetes was dependent upon an abnormal output of sugar by the liver. When it was discovered that puncture of the floor of the fourth ventricle in the neighbourhood of the vaso-motor centre gives rise to glycosuria, it was suggested that an increased vascularity of the liver, of vaso-motor origin, was the true explanation, and that all cases of diabetes were of nervous origin. This vaso-hepatic theory was for a time generally accepted, but it was severely criticised by Cohnheim and others, who pointed out that there is no clinical, or experimental, evidence of increased vascularity of the liver in diabetes, and that in any case puncture of the floor of the fourth ventricle could not bring about such an increased vascularity and accelerated blood flow as its supporters assumed. Cohnheim was inclined to take the view that diabetes depends upon the absence of a ferment which in the normal condition initiates the further destruction of dextrose. It was subsequently suggested that the liver might be supplied with glyco-secretory nerves which are independent of the vaso-motors. The absence of any satisfactory evidence of disturbances of the nervous system in the majority of cases of diabetes showed, however, that, at the most, only a small proportion could be explained on some such hypothesis. It was then suggested that the primary defect might lie in the tissues, which are, for some reason, unable to oxidise in the ordinary way the sugar brought to them, so that less is consumed than normal.

As the result of v. Mering and Minkowski's experimental work, and in consequence of the discovery that pathological changes in the pancreas exist in many cases of diabetes, the theories that had been previously held were abandoned, or modified to fit in with the view that a deficiency, or lack, of the internal section of that gland was responsible for the excess of sugar in the blood. This may be supposed to arise from some influence which the secretion normally exerts (a) upon the oxidation of sugar by the body cells, (b) upon the splitting of the sugar molecule and its preparation for oxidation by the tissues, (c) upon the storage of glycogen.

Up to the present it has not been demonstrated that the general oxidative powers of the tissues are diminished in diabetes, at least in the earlier stages. It is known that the products of protein

metabolism, urea, uric acid, &c., are approximately normal, and
that fats can be oxidised to carbon dioxide and water, that lac-
tates, inosite, mannite, and many other substances are also oxi-
dised, as in normal individuals, and that benzol is oxidised to phenol.
Moreover, in many cases of diabetes the levo-rotatory sugar, levulose,
is utilised by the organism. On the other hand, we find that in
respiratory diseases in which there is cyanosis, sugar does not
appear in the urine as we should expect it to do if diabetes depended
upon diminished oxidation alone. In phosphorus poisoning, again,
the oxidative powers of the body are distinctly reduced, yet
glycosuria does not occur. As we should expect when a con-
siderable amount of carbohydrate escapes oxidation, the quantity
of oxygen lost during respiration is lessened in diabetes in com-
parison with a healthy person, but the recent experiments of
Porges and Salomon show that the respiratory quotient of a
depancreatised dog is not different from that of a normal animal.

The fact that the administration of camphor, chloral, and
other substances that are eliminated in the urine in combination
with glucuronic acid, is followed by almost as copious an excretion
of glucuronates in diabetes as in normal individuals, has been taken
by some observers to indicate that the first step in sugar oxidation
is not interfered with. This idea is based on the assumption that
glucuronic acid is formed in the preliminary stage of the degrada-
tion of dextrose by the tissues, as a comparison of their formulæ
suggests :—

$$OH.C-(CH.OH)_4-CH_2.OH+O_2=OH.C-(CH.OH)_4-COOH+H_2O$$
$$\quad\quad\text{dextrose}\quad\quad\quad\quad\quad\quad\quad\quad\text{glucuronic acid}$$
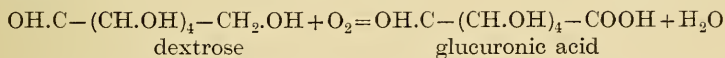
It has also been noticed that glucuronic acid may be eliminated
in diabetes without the administration of these substances, and
that sometimes it may be found in the urine when, as a result
of careful dieting, the excretion of sugar has ceased. In confirma-
tion of this view are the recent chemical experiments of Jolles,
which indicate that the oxidation of dextrose involves the inter-
mediate formation of glucuronic acid, but on the other hand the
experiments of Mendel and Jackson suggest that glucuronic acid
is only produced in the intermediary metabolism of proteins, and
not of carbohydrates at all.

Baumgarten found that the diabetic organism can oxidise sugar
after a start has been made, for depancreatised dogs were able to
complete the degradation of partly oxidised carbohydrates, such
a d-gluconic acid, d-saccharic acid, mucic acid, glucuronic acid,
succinic acid, &c. He therefore concludes that the difficulty

lies in the first attack on the sugar molecule, and suggests that this
is due to the absence of some ferment which normally initiates the
process.   The possibility that the pancreas supplies some such fer-
ment in its internal secretion has been warmly advocated by some
authors.   Lépine believes that a glycolytic, or sugar-splitting
ferment, which is supplied by the pancreas, exists, and that it is
diminished, or absent, in diabetes.   The experimental facts on
which this theory is based have, however, been severely criticised,
and it is not generally accepted.   More recently Cohnheim has
advocated the view that the pancreas supplies an amboceptor-like
substance which acts as a link between the sugar in the blood and
the tissue cells.   His conclusions have been provisionally accepted
by some authors, but his experimental work and the inferences he
draws from it have been adversely criticised by others.   Von Noorden
has suggested that the internal secretion of the pancreas is necessary
to enable the tissues to convert sugar into glycogen, which he
assumes is an essential step in the absorption of sugar into the
molecule of protoplasm.   In support of this view is the poverty
of the liver and muscles in glycogen, both in experimental and
human diabetes.

Pavy considers that the excess of sugar in the blood may arise
from two causes—first, in the milder forms of diabetes, the sugar
absorbed from the intestine is not attached and built up as it
should be, owing to the absence of an amboceptor supplied by the
pancreas ; and secondly, in severe, or what he terms " composite,"
cases, there is in addition to this a molecular disruption, attended
with the dissociation and setting free of carbohydrate that has
previously been put into combination, brought about in a similar
way to that which occurs when blood impregnated with phloridzin
is circulated in the kidneys.   This view appears to be in part
accepted by Bosanquet, who points out that the glycogenic theory,
as usually set forth, is inadequate to explain the most characteristic
feature of human diabetes, the stage at which there is a formation
of sugar from the cells of the body.   He suggests that there may
be in diabetes a poison, acting somewhat like phloridzin, which
has the power of splitting off a saccharine radicle from the proto-
plasmic molecule.   Such a substance he contends will first attack
loose combinations of sugar, such as exist in the hepatic cells,
which probably hold their glycogen attached by some mechanism
analogous to Ehrlich's side chains, and readily allow it to be split off.
The poison would equally attack at an early stage a loose combina-
tion of sugar and protoplasm such as Pavy suggests (in the lym-
phocytes), as the vehicle for the carriage of sugar to the tissues.   As,

however, the disease advances and more of the poison is formed, its activities will not be confined to these loose compounds, but it will attack the other cells of the body, breaking off from them too a saccharine radicle. Now we know that the hepatic cells can give up and resume their glycogen without injury to themselves ; such is their function. But to extract a molecule of sugar from other cells is probably impossible without destroying them. Thus it comes about that in the later stages of diabetes there is a destruction of protoplasm, with formation of acetone bodies, which are so characteristic of grave diabetes.

It has for some time been felt that even if it is conceded that the pancreas possesses an internal secretion which acts in one or other of the ways suggested, and it is allowed that the islands of Langerhans are concerned in its production, the pancreatic, like its predecessor, the nervous theory, has failed to explain all cases of diabetes. Although its more enthusiastic advocates have suggested that functional derangement of the gland may give rise to the condition without there being any structural changes, most observers are agreed that such an explanation is unsatisfactory. Many cases of diabetes are undoubtedly of pancreatic origin, some are due to nervous influences, but a certain proportion appear to be dependent upon other causes, among which are probably to be counted disease of the other ductless glands, while the part that toxic influences take in the production of glycosuria have also to be accounted for.

There is a tendency at the present time to revert to the doctrine that over-production of sugar is the main cause of diabetes, and to abandon the theory that diminished consumption is the essential element. This view is now held by a number of eminent observers, including v. Noorden. They consider that the hyperglycæmia, and consequent glycosuria, are dependent upon an excessive output of sugar by the liver, and that this may arise from abnormal stimulation, or impaired inhibition of its glycogenetic function. The stimulus to the liver may come from (1) an excess of carbohydrate food, as in alimentary glycosuria ; (2) an increased call by the tissues ; (3) hyperfunction of the supra-renals, &c. ; or (4) from the central nervous system, through the medium of the chromaffin system. Impaired inhibition may arise from (a) interference with, or suppression of, the functions of the pancreas, as in pancreatic diabetes, or (b) from interference with the controlling action of the thyroid, or hypophysis, on that organ, as in exophthalmic goitre and acromegaly. They also consider that in some cases there is probably a primary anomaly of the liver cells themselves.

According to this view the glycosuria that results from puncture
of the floor of the fourth ventricle, and that occurs in association
with disease of the nervous system, is not due to direct stimulation
of the liver through special glyco-secretory nerves, &c., as was
formerly taught, but depends upon the transmission of impulses
by the left sympathetic to the left supra-renal, whence it is trans-
mitted to the right supra-renal by the connecting nerves ; as a
result of the stimulation of these organs they function more actively,
and the increased flow of their secretion in its turn brings about
an excessive output of sugar by the liver.   Cannon and his asso-
ciates have shown that fright, anger, asphyxia, and the strong
stimulation of sensory nerves all cause an increased secretion of
epinephrin, and consider that the glycosuria that results from worry
and mental strain may be contributed to by the same cause.
Von Noorden suggests that a number of toxic influences may act
in a similar way, the glycosuria to which they give rise being partly
the result of an action they exert on the diabetic centre in the
medulla, and partly an effect of their stimulating action on the
supra-renals, or the sympathetic nerves controlling them, thus in
any case bringing about hyperfunction of the chromaffin system,
with a consequent over-production of sugar by the liver.

This conception of glycosuria correlates in a much more satisfac-
tory manner than has previously been possible the experimental
facts, and all that is known of the etiology of diabetes.   At the
same time it destroys the idea of a sharply defined disease, and
substitutes a system complex, liable to arise from a variety of
causes.   According to this view the normal metabolic level is
maintained by the balance existing between a number of mutually
controlling forces.   Should the balance be disturbed a lowered
tolerance for dextrose, a temporary, or intermittent, glycosuria, a
persistent glycosuria, or a typical diabetes may result, according to
the nature, extent, and permanency of the disturbance.   If this be
so, it follows that there is no such thing as a non-diabetic glycos-
uria, with possibly the exception of the so-called renal diabetes,
and that any difference is merely of degree, and not of kind.

Magnus Levy in his Cartwright Lectures maintained on the con-
trary that the conception of diabetes as a concrete unity is justi-
fiable, since the metabolic disturbance and its intensity dominate
the pathological process.   He regards a primary disturbance of
the sugar-splitting process as the essential factor in severe cases,
and considers that complete acceptance of the view that diabetes
arises only from an increased formation and mobilisation of sugar
must lead to the conclusion that proteins and fats are normally

transformed into sugar. There is, however, no justification for such a conclusion. He grants that increased mobilisation co-operates in the production of diabetes, and also that, in a certain sense, there is an increased formation of new sugar, but maintains that this increase in sugar formation and mobilisation is only a secondary consequence of disturbed combustion. He points out that in spite of the fact that sugar is wasted in the body, those organs which need it do not cease in their demands to be supplied. The liver and other producers of new sugar strive to meet their requirements, forming it, and sending it to other tissues, but without any advantage to the organism. This disturbance in the utilisation of sugar increases in severe cases with the lapse of time, but it need never be absolute, some of the newly formed sugar being catabolised. According to this theory the muscles, as the principal organs concerned in the combustion of sugar, would come more into the foreground of the picture, the mobilising organs, such as the supra-renal capsules and the thyroid, taking a secondary place. Levy holds that a form of diabetes exists, in which the muscle system alone is involved, and that, even if the attempts that are being made to isolate the hormone of the pancreas, and to prove the co-operation of the pancreas and muscles in carbohydrate metabolism are successful, the solution of the problem lies in the direction he indicates.

Pathology of the Symptoms and Complications of Persistent Dextrosuria.—Whatever may be the explanation of the imperfect utilisation of sugar by the organism in chronic glycosuria, there can be no doubt that most, if not all, the symptoms can be referred to interference with the normal source of energy that the loss of sugar in the urine entails, to the high sugar-content of the blood, or to the presence of products of imperfect metabolism in the blood and tissues. The manner in which the products of imperfect and abnormal metabolism bring about the symptoms of acid intoxication have already been considered in connection with acidosis and diabetic coma, it now only remains, therefore, to briefly refer to the way in which it is believed that others of the main symptoms and complications are produced.

The carbohydrates of the food are the most important source of energy for the body, and it consequently follows that if a patient on an ordinary diet is unable to make use of a part or the whole of the carbohydrate he consumes, but passes it in his urine in the form of dextrose, there is a corresponding loss of energy-forming material which normally would be used for the production of power and heat. Each gram of dextrose lost to the organism in this way

R

represents about 4 Calories, so that if 100 grams are excreted daily
there will be a loss of 400 Calories, or sufficient heat to raise 4000
grams of water through 100° C.[1] A man of average size doing
light work requires from 2500 to 3000 Calories a day, so that such a
loss is equivalent to nearly one-sixth of the total caloric expenditure
of the body, and is inconsistent with the maintenance of a good
state of nutrition. So long as the patient is able to digest and
absorb enough food to compensate for what he loses in his urine
his weight and powers of work may not seriously suffer, but even-
tually a time arrives when this is no longer possible, and the tissue
fats and proteins are used to supply the energy required, with the
result that there is a gradual *loss of weight* and increasing *weakness*.
Eventually, if the patient is not meanwhile carried off by some
intercurrent disease or complication, he dies from what is practically
acute starvation, for all the available fat and proteins having been
exhausted sufficient energy to maintain the vital functions is no
longer obtainable.

The defective power of metabolising carbohydrates is no doubt
the explanation of the *voracious appetite* of many diabetics. In
some instances enormous quantities of food are consumed, and
yet the patient is hungry. In a case quoted by Quintard a diabetic
of sixty-two took $4\frac{1}{2}$ kilograms (10 lb.) of solid food a day, and Külz
records a case in which between 5 and 6 kilograms (11 to 13 lb.)
were consumed daily. Although the food for a time appeases the
pangs of hunger in the stomach, the cravings of the tissues remain
unsatisfied, since the chief source of energy, the carbohydrate it
contains, cannot be made use of. It is only when an amount of
protein and fat sufficient to supply the energy required is added
to the diet, and the unusable carbohydrates are eliminated, that
the polyphagia disappears. The excess of food taken by patients
whose diet has not been arranged on scientific lines is apt to cause
dilatation of the stomach and set up *gastritis*, which add further
complications to the condition.

Both the *polyuria* and *thirst* that are so frequently found as
symptoms of diabetes are referable to the excess of sugar in the
blood and its excretion in the urine. Experiment has shown that
if the sugar-content of an animal's blood be increased there is a
prompt rise in the quantity of urine passed, probably because an
additional amount of fluid is needed by the epithelial cells of the
kidneys to enable them to separate the sugar and eliminate it from
the body. The blood thus tends to diminish in volume, but this

[1] A large Calorie is the amount of heat required to elevate the temperature
of 1000 grams of water 1° C. (See p. 284.)

tendency is counteracted by a withdrawal of water from the tissues. In some obscure way this loss of water by the tissues affects the nervous system, calling forth the sensation of thirst. Another factor that probably assists in the production of this sensation is the presence of chronic gastritis from which so many diabetics suffer.

Some of the complications met with in diabetes depend upon the fact that the saccharine urine forms an excellent medium for the growth of micro-organisms, while others are due to the altered chemical and physical characters of the blood and tissues. The *pruritis, dermatitis, and eczema* that develop in the parts about the urinary and genital tract, especially when strict cleanliness is not observed, arise from the irritation produced by the growth of fermentative organisms in the urine clinging to the parts. Occasionally the urine undergoes similar fermentative changes within the bladder, giving rise to *pneumaturia*, or, if the urine becomes infected with coliform and other organs, to *cystitis*.

The excess of sugar in the blood seems to exercise a deleterious influence upon the tissues of the body, which is especially seen in the *failure of wounds to heal*, and in the tendency of slight injuries to lead to extensive tissue *necrosis and gangrene*. That this tendency to tissue disintegration and necrosis is probably dependent upon the hyperglycæmia is suggested by the fact that any measures which result in a reduction in the quantity of sugar in the blood favourably influence the tissue changes, but since the amount of tissue destruction does not appear to bear any direct relation to the intensity of the hyperglycæmia, it is possible that some unknown toxic influence that accompanies the latter may also be involved. The increased osmotic pressure of the blood from the excess of sugar it contains possibly also plays some part in the tissue changes, but with the exception of Pusey's experiments, which suggest that an alteration in osmotic pressure is an important factor in the production of *cataract*, no observations on this subject appear to have been carried out. The tendency to gangrene is contributed to, in some instances, by a defective blood supply consequent on arterio-sclerotic changes in the blood-vessels.

The marked susceptibility of diabetic subjects to various *infections*, and particularly infection by pyogenic organisms and tubercle bacilli, is generally attributed to the abnormally high sugar-content of the blood, which might favour the growth of various bacteria, or possibly neutralise the bactericidal and other anti-infectious powers of the blood. There is no experimental evidence in favour of the view that increasing the amount of sugar in the blood, within reasonable limits, produces either of these

effects. Thus Handmann found that the addition of glucose to the blood to the extent of 0·5 per cent. to 1 per cent. does not render it a better culture medium for staphylococci than normal blood ; further, that the addition of sugar to the blood does not diminish its bactericidal power for staphylococci, nor does it reduce the opsonic power of the serum with reference to this coccus. It may be noted, however, that Sweet in pancreatectomised dogs found that the serum in the later stages loses its bactericidal power, especially with reference to colon, typhoid, and dysentery bacilli, and also to a large extent its haemolytic power. Da Costa and Beardsley observed a distinct diminution in the opsonic power of the serum in diabetics for streptococci, staphylococci, and tubercle bacilli. They did not, however, determine whether this diminution in opsonic power was due to a diminution in the thermostabile opsonic element or in the thermolabile element, or complement ; neither did they make repeated observations on the same patients over long periods of time so as to determine what relations, if any, exist between the changes in the opsonic power of the serum and the occurrence of complications due to the usual diabetic infections. It has been suggested that the diminished alkalinity of the blood in diabetes reduces its anti-infectious power, but this possibility has not been investigated with sufficient thoroughness to develop any definite facts. It has also been urged that there is no change in the general anti-infectious powers of the blood and other fluids in diabetics, but rather a local loss of resistance concerning the exact nature of which no explanation at all has been advanced. The latter view is based largely on the fact that diabetic patients do not seem to be much more liable to general infections and to miliary tuberculosis than other individuals. It would seem, however, that the observation by Sweet that the serum of pancreatectomised dogs loses its bactericidal power, and the observation by Da Costa and Beardsley of the diminution in opsonic power of the serum of diabetics, point distinctly to general diminution in the anti-infectious powers in diabetes.

The inability of the tissues to satisfactorily metabolise carbohydrates probably leads to impaired nutrition of the cells, and this, with the injury due to the accumulation of deleterious waste products, may predispose them to degenerative changes and the attacks of micro-organisms, thus accounting for the tendency to *furunculosis* and other forms of suppurative infection, notably *phlegmons and carbuncles*, which may run on to gangrenous processes. The complication of pulmonary tuberculosis with *gangrene of the lung* is possibly explicable on similar lines.

Diagnosis.—In the diagnosis of glycosuria three main errors have to be guarded against—(1) the possibility of not recognising a small amount of sugar in the urine, owing to the masking effect of creatinin, albumen, ammonia, &c., when reliance is placed on Fehling's and similar reduction tests alone ; (2) the reverse mistake, which appears to be more common, namely, attributing a reduction due to uric acid, &c., to the presence of sugar ; (3) mistaking a reduction due to pentoses, lactose, levulose, and more particularly to glucuronic acid compounds, for that of dextrose. The precautions necessary to avoid these errors, and the methods by which the commonly occurring reducing substances in the urine can be differentiated, have already been fully dealt with ; but I think it is necessary to again draw attention to them, as my experience suggests that too much reliance is often placed upon the results of a single test, and more especially on the findings obtained with Fehling's solution. During five consecutive years I had sent to me thirty-seven cases with a diagnosis of " diabetes " in which dextrose was absent from the urine. In three the sugar was found to be dextro-rotatory l-arabinose, apparently of alimentary origin, in one case inactive arabinose appeared to be present, but I could not obtain sufficient material to thoroughly study the case, small quantities of a sugar resembling maltose were met with in two cases, in two women lactose was found, the reduction in eighteen appeared to be due to glucuronic acid compounds, and in six was probably dependent upon uric acid or similar reducing substances, for no trace of sugar could be detected with the phenylhydrazin and other tests ; in five cases I failed to obtain any reaction with Fehling's solution, &c., and could only refer the results reported to me to faulty technique on the part of the observer.

In the present state of our knowledge it is rarely possible to determine with certainty the underlying cause of a persistent glycosuria in any particular case. A minute examination of the family history, the previous health of the patient, his mode of life and surroundings, and his present symptoms and physical signs will sometimes suggest a probable cause, but it is not often that a definite conclusion can be come to. A diagnosis is most readily arrived at when evidence of pancreatic disease is found. The possibility of this may be suggested by the history of the case, but it is only reached with certainty by a thorough qualitative and quantitative examination of the fæces and urine.

When making an examination of the fæces in a case of suspected pancreatic disease my procedure is to first notice the naked-eye characters, and then make a careful microscopical investigation

of specimens taken from various parts of the sample. A chemical analysis for trypsin, amylopsin, stercobilin, and occult blood is then undertaken, and finally the percentages of total, "saponified," and "unsaponified" fats, and the proportion of inorganic ash are quantitatively determined. It will here be only necessary to refer to the method I employ for the estimation of fats, the other tests being described in their appropriate places subsequently.

Rapid Estimation of the Fat-content of the Fœces (Cammidge).—For this purpose I have adopted a procedure, which, while much more rapid than Soxhlet's process, gives results which are quite satisfactory for clinical purposes. The method is an adaptation of the Schmidt-Stokes process of estimating fat in milk. It may be briefly described as follows. Two clean, dry, Schmidt-Werner tubes, labelled A and B, and provided with a 10 c.c. mark are taken. Into the lower bulb of each is introduced an accurately weighed quantity of the thoroughly dried and finely powdered fæces ; I usually employ about half a gram. The residue on the watch-glass used for weighing, and on the sides of the short-necked funnel with which the powder is introduced into the tube, is washed down with a fine jet from a wash bottle, which for the A tube contains hydrochloric acid (1 in 3), and for the B tube distilled water. The sides of the tube are also washed down until the whole of the sample is collected into the lower bulb, and the 10 c.c. mark is reached. The A tube is then heated in boiling water for a quarter of an hour, occasionally rotating it so as to well mix the contents. After it has cooled, both tubes are filled to the 50 c.c. mark with ether, securely corked, and inverted thirty or forty times, allowing the whole of the solid material to run through the ether each time. Each tube is then rotated between the hands, fixed in an upright position, and left undisturbed for half an hour or more, so that the whole of the solid residue may be brought into the lower bulb. Considerable care is required in this part of the operation, or a perfectly clear supernatent layer of ether, free from solid, may not be secured. With a pipette exactly 20 c.c. of the ethereal extract is drawn off from each tube and delivered into two CO_2-flasks of known weight, the amount of ether remaining in each tube being noted. The ether in the flasks is evaporated off, the residue dried by heating on a water-bath, and the flasks again weighed. From the amount of extract yielded by the 20 c.c. of ether, and the quantity of ether left in the tubes, the total amount obtained from the weight of dry fæces used may be calculated, and from this the percentage in the stool determined. For convenience of reference I am in the habit of describing the yield from the A tube as " total fat," that from the B tube as " unsaponified fat," and the difference between the two as " saponified fat," and this is the meaning I shall attach to these terms when employing them subsequently.

The solid residue in the B tube can be used for detecting *stercobilin.* For this purpose it is filtered off, extracted with acid alcohol, the acid

neutralised with ammonia, and an equal volume of 10 per cent. zinc acetate in alcohol added. The precipitate which forms is removed by filtration, and the clear filtrate examined against a black background for the green fluorescence which indicates the presence of stercobilin. The intensity of the colour varies with the amount of pigment present, so that by always using approximately the same quantities of fæces and of the reagents, any marked variation from the normal is readily detected.

Lancereaux, in 1877, claimed that diabetes associated with disease of the pancreas was always accompanied by marked wasting (dia'ète maigre), and was further characterised by the brusqueness of its onset, the gravity of the symptoms, and the rapid progress of the disease, while that in which there was no marked loss of flesh (dia'ète gras), and which ran a longer and more benign course, was due to some other cause. Although it is true that some cases of pancreatic diabetes do run a rapid course, and are exceedingly grave from the first appearance of the symptoms, this, in my experience, is rather the exception than the rule. I have had the opportunity of investigating a number of cases of diabetes of undoubted pancreatic origin in which there has been no marked loss of flesh, the onset had been insidious, and the general health was good several years after the discovery of the glycosuria. It is now generally acknowledged that, as a rule, the character of the onset and symptoms afford no evidence on which a reliable opinion can be based as to the origin of the disease.

Fæces.—There appears to be a general impression that disease of the pancreas is always accompanied by an alteration in the appearance and physical characters of the fæces. This is far from being the case, for apart from those cases where there is associated biliary obstruction, the pale bulky motions, of a white or oily appearance, described in text-books, are very uncommon, being only seen in very advanced cases. When present they present characters which, to the experienced eye, distinguish them from those met with in other diseases. They are whiter, more glistening, and sometimes contain masses of yellow, oily, undigested fat. Their smell, like that of rancid bacon, is very typical. Their lack of colour is partly due to the excess of fat they contain, particularly to the free fatty acid crystals, and partly to a reduction of the normal colouring matter to a colourless derivative through the action of anærobic bacteria.

The reaction of the normal fæces is amphoteric, faintly alkaline, or very faintly acid, but in pancreatic diseases it is frequently acid, sometimes very markedly so. With intestinal catarrhs and biliary

obstruction the stools are usually alkaline, so that when a pancreatitis is due to intestinal or biliary trouble the acid reaction may be masked by the alkalinity arising from this cause. In some cases of pancreatic disease the acid reaction is so marked that it produces considerable irritation of the bowels, and I have met with cases of pancreatic glycosuria in which this was so great that the patient's life was only made bearable by washing out the rectum several times a day with an alkaline solution. Confirmatory evidence of the presence of an intestinal catarrh is given by an excess of inorganic ash in the fæces, the excess being more marked when the colon, as well as the small intestine, is involved.

Fats:—One of the most important and characteristic functions of the pancreas is to prepare the fats of the food for absorption by splitting them into fatty acids and glycerine ; the former combine with bases in the intestine to form soaps, and these, in the presence of bile, are absorbed by the epithelial cells of the intestinal wall. In a healthy individual, taking an average amount of fat, from 20 to 25 per cent. of the dry weight of the fæces consists of unabsorbed fat, and of this about an equal amount—viz. between 8 and 12 per cent.—consists of " saponified " and " unsaponified " fat. Any disease of the pancreas interfering with its digestive powers, such as advanced cirrhosis or cancer, leads to an increase in the proportion of unabsorbed fat, and the greater part of this is usually found to be in the unsaponified form. On the other hand, with diseases of the intestine or obstruction of the bile flow, where fat absorption is interfered with, the saponified fats are found to be in excess. If there is both interference with the digestive functions of the pancreas and biliary obstruction or intestinal disease, the relation between the saponified and unsaponified fats will depend upon the relative extent and intensity of the two conditions. In interpreting the results of an analysis of the fæces for fats, one must therefore take into account the other indications given by the clinical symptoms and by an analysis of the urine and fæces. In the early stages of chronic pancreatitis, when as yet the disease is of the purely catarrhal type, there is probably an increased flow of pancreatic juice analogous to the salivation seen in parotitis, hence fat digestion is often more active than usual, so that a low reading of total fat is obtained on analysing the fæces, and an excessive proportion of the fat is found to be in the saponified form. When, however, the inflammatory changes have persisted for some time, and there is well-marked cirrhosis, analysis of the fæces may show as much as 50, and rarely, even 80, per cent. of unabsorbed fat, and

as a rule the unsaponified are in excess of the saponified fats. With cancer of the pancreas the total fat content of the fæces is always high, averaging 71 per cent. for the cases I have examined.

Intestinal diseases interfering with absorption may, however, also show 50 to 60 per cent. of unabsorbed fat in the stools. Other conditions associated with an excess of fat in the fæces which must be taken into account when investigating the functions of the pancreas are disorders of the stomach, which prevent the breaking down of the connective tissue septa binding the fat together, and a diet containing an abnormal amount of fat, particularly when this is of a kind that is digested with difficulty. The mere presence or absence of an excess of fat in the fæces therefore, although suggestive, is by no means conclusive evidence of the existence or not of disease of the pancreas. In a considerable number of cases of pancreatic diabetes no alteration in the fat content of the stools can be discovered. The relation between the saponified and un- saponified fats is a much more important diagnostic point, but even here the modifications produced by the causes I have men- tioned must be constantly borne in mind. Should an excess of fat, and particularly an abnormal proportion of unsaponified fat when the patient is on an average diet, be reduced by the ad- ministration of fresh pancreas, or an active preparation of the gland, by the mouth, the presence of pancreatic disease is rendered more probable.

The digestion of proteins is another important function of the pancreas, and the appearance of numerous undigested muscle fibres in the fæces tends to indicate serious pancreatic mischief, but it is not justifiable to conclude that the functions of the pancreas are interfered with from this alone, for, excluding their presence from an excess of meat in the food, undigested muscle may also be found in cases where, owing to increased peristalsis or putrefactive changes, leading to secondary diarrhœa, the food is hurried through the intestine before it has had time to be digested. Defective gastric secretion may also lead to imperfect digestion of muscle, as the connective tissue binding the fibres is not dissolved.

According to Schmidt cell-nuclei are not attacked in the stomach but are digested by the pancreatic secretion, hence the discovery of well-preserved cell nuclei in the muscle fibres in the fæces in- dicates pancreatic insufficiency. For the purpose of his test small cubes of meat, enclosed in gauze bags, and previously hardened in alcohol, are swallowed by the patient and subsequently recovered from the fæces. The contents are washed, stained, and examined microscopically.

Müller has shown that the normal fæces contain a tryptic
ferment which can be detected by allowing an extract of the stool
to act upon a serum plate at body temperature, and Schlecht has
made use of this test as an indication of the functional activity of
the pancreas. Subsequently Gross and Heiberg substituted an
alkaline solution of casein for the serum plate, and test the amount
of digestion that has taken place by precipitating the casein that
remains unaffected with dilute acetic acid. These tests are of con-
siderable value in showing serious interference with the digestive
functions of the pancreas, such as is met with in advanced cirrhosis
of the gland and in cancer associated with an established glycos-
uria ; but they do not distinguish the early pre-glycosuria stages
of chronic pancreatitis, which are most amenable to treatment.
Further, the value of the test may be interfered with by the partial
digestion of the casein by erepsin.

In a paper read at the International Congress of Physiologists
held at Brussels, Boldireff stated that the introduction of a large
amount of oil into the stomach causes relaxation of the pylorus
and the reflux of bile and pancreatic juice. Taking advantage of
this observation, Volhard suggested a clinical method of testing
the functional activity of the pancreas. By means of an œsophageal
tube 150 to 200 c.c. of olive-oil are introduced into the stomach,
and withdrawn again in a quarter to three-quarters of an hour.
The recovered liquid is then tested for the presence of trypsin, as
in Gross' test. It has been found that when the stomach contents
are unusually acid the findings are positive only when the acidity
has been neutralised, so that it is advisable to administer half a
teaspoonful of burnt magnesia, or some other alkali, before giving
the oil. Volhard obtained satisfactory results with normal indi-
viduals, and with a few cases of advanced disease of the pancreas
that he examined. Lewinski, who investigated twenty-nine cases,
reports that the absence of trypsin is an important sign of pan-
creatic insufficiency or of a mechanical obstruction to the passage
of pancreatic juice into the stomach. Koziczkowsky carried out
the test with eighty patients suffering from a variety of diseases,
using 150 c.c. of oil or 250 c.c. of cream, and obtained a more or
less marked reaction for trypsin with seventy-two. In eight no
trace could be discovered, and these were found to be suffering
from inoperable cancer of the stomach, pernicious anæmia, severe
liver affections, or diabetes. The smallest proportions of trypsin
were met with in patients with catarrh of the small intestine,
cholelithiasis, and diabetes insipidus. He concludes that a series
of tests is advisable, that the constant absence of trypsin points

to severe functional disturbances of the pancreas, and that the presence of large amounts exclude advanced pancreatic mischief.

Ehrmann has recently suggested a modification of this test. It is based on the fact that a neutral fat is not split by anything but the fat-splitting ferment of the pancreas, so that by adding a stain which only acts on the fatty acids to the recovered stomach contents their presence is rendered evident.

The patient is given a test breakfast of 30 grams of rice starch dissolved and warmed in a glass of water, a trace of salt is added, and 75 grams of commercial palmin, liquefied by heat, stirred in. After two or two and a half hours the stomach contents are siphoned out, and a portion mixed with an equal part of a mixture of 90 parts of petroleum, benzin, and benzol to 100 parts. After the mixture has been well shaken, the supernatent ether layer is decanted and mixed with an equal part of a 3 per cent. solution of copper acetate in water. The ethereal layer then assumes a bright green tint in proportion to the content of fatty acid.

Starch.—Reduction or failure of the pancreatic secretion might be expected to lead to impaired digestion of starchy foods and the appearance of an excess in the fæces. Observations made by various observers have shown, however, that only a small proportion, or none at all, of the starch of the food is excreted in the fæces in an unchanged condition in cases where these conditions exist. The discovery of unaltered starch granules in the stools is more commonly an evidence of a catarrhal condition of the upper part of the intestine, causing the food to be hurried along at an abnormally rapid rate, than of pancreatic disease.

Attempts have been made from time to time to estimate the functional activity of the pancreas by determining the amount of diastase in the fæces and urine, but as a rule with no very satisfactory result. For this purpose the modification of Robert and Strassburger's method devised by Goiffon and Tallarico may be followed.

A 1 per cent. solution of starch is mixed with an equal part of a 10 per cent. solution of the stool, neutralised, and filtered. The mixture is kept at a constant temperature of 38° C., and at regular intervals a drop is brought in contact with a drop of iodine solution. When it ceases to stain blue the digestion of the starch has been complete. The stool should be fresh, and there should not be the slightest admixture of urine. It is often enough merely to mix the stool and starch solutions in a test-tube, heat in hot water, and apply the iodin test. If there is abundant amylase present, the starch will be digested in five minutes.

When greater precision is desired, it is better to use a 5 per cent. solution of the fæces, and dilute further with an acid solution made with 10 parts normal solution of hydrochloric acid, 5 parts sodium chloride, and 1000 parts water. About 5 c.c. of the stool are ground in a mortar with 50 c.c. of water. The mixture is poured into a test-tube, and all above the 50 c.c. mark represents the volume of fæces. Water is then added at the rate of 20 c.c. for each cubic centimetre of fæces. In another test-tube are placed 2 c.c. of the 1 per cent. starch solution, 5 c.c. of the above acid solution, and the whole is heated in water toward 40° C. Then, noting the time, 2 c.c. of the solution of fæces are added, and at regular intervals a drop is added to a drop of the official compound solution of iodin until all bluish or reddish dis-coloration ceases. For extreme precision, the index of the findings can be the time required for digestion of the starch, divided by the dry weight per hundred of the stools.

The discovery of Roger and Simon that saliva, inhibited in its activity by gastric juice, recovers its digestive powers when trans-ferred to an alkaline medium and a little unmodified saliva or pancreatic juice is added, has been applied by Fedeli and Romanelli to the testing of the functions of the pancreas.

To 1 c.c. of the patient's saliva they add 5 c.c. of gastric juice, or an equal quantity of 2·5 per cent. HCl; shake the mixture, and leave it to rest for half an hour; then add 4 c.c. of a 1 per cent. solution of carbonate of soda, so as to render the mixture slightly alkaline. They next add 20 c.c. of a 10 per cent. starch paste, and place the whole in thermostat at 37° C. for two hours, repeatedly shaking. The amount of sugar formed is then estimated. The next stage consists in adding to the above mixture 10 c.c. of an aqueous solution (1 in 4) of fæces, and leaving the whole in the incubator for twelve hours, and then estimating the sugar formed. The difference between the two estimations of sugar represents the degree of pancreatic functionality. To show that this was not due to other constituents of the fæces, the authors tested with bile and saccus entericus, and found that they both gave negative results.

Even when an examination of the fæces points to the existence of pancreatic insufficiency it does not necessarily follow that disease of the pancreas exists and is the cause of the glycosuria, for a healthy gland may fail to function because it has not been stimu-lated into activity in a normal manner. Modern research has shown that the processes of digestion are intimately linked together and form a continuous chain, each step in the series calling forth that which succeeds it, so that any inefficiency or break in the chain disorganises the whole process. For the normal perform-ance of the digestive functions of the pancreas it is necessary (1) that the stomach should supply a stimulus in the shape of an acid

chyme which will act upon the duodenal mucous membrane, giving rise to the " secretin " that will rouse the pancreas into activity ; (2) that the pancreas itself should be able to respond and secrete the necessary ferments ; (3) that bile should reach the intestine to aid in digestion and absorption ; and (4) that the intestine should secrete the " enterokinase " by which the inactive proteolytic ferment, trypsogen, of the pancreatic juice can be converted into active trypsin. Failure of any one of these may lead to symptoms of pancreatic insufficiency.

1. We will first consider pancreatic insufficiency dependent upon gastric troubles. If the secretion of hydrochloric acid by the stomach is deficient, or absent, the pancreas will act imperfectly, or not at all, for although fats and a few other substances appear to have the power of forming secretin from the intestinal mucous membrane, their activity in this respect is much less than that possessed by hydrochloric acid. In such cases, too, the pylorus opens at an abnormally early stage in digestion, so that the intestine is faced with the problem of dealing with materials in which there is not only a deficiency or absence of pancreatic ferments, but which have also been imperfectly prepared for the action of any ferment that may be there, with the result that the symptoms of " intestinal " indigestion ensue, and an examination of the fæces shows an abnormal amount of undigested food material. The absence of hydrochloric acid also tends to favour the growth of an abnormal intestinal flora. If, on the other hand, an excess of . acid is poured out by the stomach, the pancreas may for a time be able to cope with it by secreting a corresponding amount of its alkaline juice, but the over-stimulation of the gland leads to degenerative changes, which are indicated by a marked pancreatic reaction in the urine, and eventually these bring about a diminished secretion. If the hyperchlorhydria continues it will cause an abnormally acid condition of the intestinal contents, and so interfere with the activity of any pancreatic ferments that may be present, for these are quickly destroyed by free mineral acids, so that pancreatic insufficiency is again brought about but in an altogether different way. In either case the only certain way to diagnose the cause of the condition is by the administration of a test meal.

2. The part the bile takes as an adjunct in the digestion and absorption of fats is well known, but it also appears to exert a very material influence on the digestion of proteids. It is therefore very necessary for satisfactory digestion that bile should be present in the intestine, and its absence is often a contributory factor in the production of pancreatic insufficiency.

3. Another important adjuvant to the digestive action of the pancreatic juice is enterokinase, a ferment present in the succus entericus, which has the power of augmenting the activity of the pancreatic ferments, and more particularly the proteolytic, to a striking degree. This " ferment of ferments " is secreted by the intestinal mucous membrane, chiefly in the duodenum, apparently through the stimulus afforded by the pancreatic juice. In certain diseases of the intestine it is probable that its formation is interfered with, and there may consequently be defective pancreatic digestion, not from true pancreatic insufficiency, but from a lack of the activating ferment. The diagnosis of such a condition is not easy, and its presence can only be inferred when an analysis of the fæces reveals imperfect digestion, particularly of proteids, and there is no evidence of pancreatic disease or true pancreatic insufficiency.

4. The fourth variety of pancreatic insufficiency is the true form in which, owing to lesions of pancreas or obstruction of the ducts, there is imperfect digestion from diminution or absence of the pancreatic ferments. This is seen in advanced cases of cirrhosis of the pancreas, in some cases of pancreatic calculi and cysts, in cancer of the pancreas, particularly of the head of the gland, and in occlusion of the ampulla of Vater by gall-stones, growths, or stricture.

The presence of *lecithin* in the fæces is said to indicate disease of the pancreas, but as traces are found in health, and the quantity present is liable to be increased from other causes than interference with the functions of the pancreas, it is not a sign of much value.

Sometimes chronic pancreatitis giving rise to glycosuria is the result of invasion of the pancreas by growths or ulcers of the stomach or intestine, and the discovery of *occult blood* in the fæces is suggestive of one or the other. If the blood is found in every specimen on four or five successive days it points to a malignant growth, whereas its intermittent presence is suggestive of a duodenal or gastric ulcer which may be invading the pancreas, or simply be associated with a catarrhal condition of the upper part of the intestine to which the pancreatitis is secondary. Occult blood is also found very constantly in cases of cancer of the pancreas and with growths of the common bile-duct or gall bladder, which may invade the pancreas and cause glycosuria. Occasionally it is met with in advanced pancreatitis without actual alteration of the intestinal mucous membrane, probably as a result of the hæmorrhagic tendency.

The Urine.—As complete an analysis as possible of the urine

should of course be made in all cases of glycosuria, but this is particularly important when it is suspected that the presence of sugar is dependent upon disease of the pancreas. The changes met with in the urine in association with pancreatic disorders depend partly upon the altered conditions existing in the intestinal tract, and partly upon the perverted chemistry of the body. The chief alterations that have been observed are in the amounts of indican, ethereal sulphates, total nitrogen, uric acid, phosphates, calcium oxalate, urobilin, and bile, and according to some observers the occasional presence of maltose and pentoses. Sahli's test and the so-called " pancreatic " reaction may also be of some assistance in arriving at a diagnosis.

Sulphates and Indican.—In the normal condition about a tenth of the total sulphates of the urine occur in combination with aromatic alcohols (indoxyl, skatoxyl, cresol, phenol, &c.), and are consequently spoken of as ethereal, or conjugate, sulphates. While their amount is subject to great and inexplicable variations, the quantity present in any case may be considered as a fairly accurate index of the extent to which absorption of the products of intestinal decomposition that can pair with sulphuric acid is taking place. It has been stated that when the flow of pancreatic juice into the intestine diminishes, or ceases, the proportion of ethereal sulphates falls, probably because less indol, skatol, &c., are formed in the intestine. Several observers have, however, found an increase in both the conjugate sulphates, and in the indican excretion in such cases. My own observations during the past ten years have given such varied results that I have come to the conclusion that no reliance can be placed upon estimations of the ethereal sulphate excretion or indican output, in the diagnosis of pancreatic disease, and that an excess of both indicates rather an associated enteritis and hepatic insufficiency.

Azoturia and Endogenous Uric Acid.—An excess of nitrogen is said to occur in the urine in diseases of the pancreas, but as this also exists in all forms of diabetes, it is of no assistance in the differential diagnosis. Rosenberger states that in pancreatic diabetes there is a diminished excretion of uric acid when the patient is placed on a purin-free diet, and suggests that this may be of use in the diagnosis of the condition.

Phosphates.—According to Dominicus, an increase in the excretion of phosphoric acid is characteristic of pancreatic lesions, but since the chief source of the phosphoric acid in the urine is the food, the nature of this will largely control the output. It is therefore advisable that, if a diagnosis is attempted by this means,

the patient should be placed upon a milk diet and the excretion of phosphates be compared before and after the administration of an active pancreatic extract.

Calcium Oxalate.—From an early stage of my work on diseases of the pancreas I was struck by the frequency with which deposits of calcium oxalate crystals were found in the urine in chronic pancreatitis. Subsequent observation showed that this was due to an increased excretion, and did not depend merely on an alteration in the physical characters of the urine that favoured the deposition of the crystals. It has long been known that glycosuria sometimes follows continued oxaluria, and that, in some cases of diabetes, a diminution in the output of sugar is associated with an increase in the oxalate deposit. Helen Baldwin found that in dogs the excretion of oxalates is increased by the administration of sugar, and Ssobolew states that overfeeding animals with carbohydrates gives rise to changes in the islands of Langerhans, so that it is not improbable that oxaluria may in some way be due to a disturbance of metabolism arising from pathological changes in the pancreas.

Bile and Urobilin.—The presence of bile in the urine shows that there is some obstruction to the free flow of bile into the intestine and, if associated with glycosuria, points to the presence of gall-stones in the pancreatic portion of the common bile duct, malignant disease of the head of the pancreas, or a growth of the common duct, ampulla of Vater, duodenum, &c., involving the pancreas. In my experience urobilinuria is most frequently associated with infection of the bile ducts, and its presence points to floating gall-stones in the common bile duct, or to an ascending infection from the intestine, which may also involve the pancreatic ducts and pancreas.

Maltose and pentoses will be considered under maltosuria, and pentosuria, respectively, but it may be stated here that their presence in the urine in pancreatic affections is so exceedingly rare that it may be a mere coincidence, and is of no practical diagnostic value.

A whole series of special tests have been devised with the object of elucidating the state of the functions of the pancreas.

Sahli's Test.—This test of the functional activity of the pancreas depends upon the fact that if iodoform, enclosed in a gelatine capsule hardened with formalin, is given by the mouth, it passes through the stomach unchanged, but is dissolved by the pancreatic secretion, so that iodine appears in the urine in from four to eight hours after its administration. The presence of the iodine is detected by adding a drop of nitric acid and shaking with

chloroform, when the characteristic violet colour is seen in the chloroform extract. If the digestive functions of the pancreas are impaired, or have ceased, the urinary reaction is delayed, or absent. So many sources of error attach to this method, notably the difficulty of properly adjusting the hardness of the capsules, and the dependence of the test on the motor-efficiency of the stomach, that it is not often used.

Other observers have employed pills containing potassium iodide, sodium salicylate, or methylene blue, coated with keratine, hardened gelatine, or wax.

The So-called " Pancreatic " Reaction in the Urine.—The " pancreatic " reaction was first described in my Arris and Gale Lecture at the Royal College of Surgeons in 1904. In 1906 I published an improved method by which some of the defects of the original test were overcome, and the result was, to a certain extent, made independent of the experience of the observer.[1]

I have now been using this method almost daily for six years, and have examined nearly 3000 urines by means of it, but I have had no reason to alter the opinion that I have repeatedly expressed that, although *not pathognomonic*, the results of the test, when considered in conjunction with the clinical symptoms and an analysis of the fæces, are clinically useful. A positive reaction in my experience is usually associated with some functional disturbance of the pancreas ; generally the result of active degenerative changes of an inflammatory character. Both clinical observations and experiments on animals have shown that malignant disease and cirrhosis of the pancreas are not, as a rule, accompanied by a positive reaction, for the diagnosis of these conditions reliance must be chiefly placed on an analysis of the fæces. The literature relating to the " pancreatic " reaction in the urine is now so extensive that to adequately review it would occupy a very considerable space, and I can here merely state that my observations and conclusions have been borne out by a considerable number of independent observers, and that, if the reactions and controls are carefully carried out, the information obtained is of distinct diagnostic value.

Taking advantage of the fact that the reaction is due to a substance that is not readily fermented by yeast,[2] a modification of the original test was devised for application to urines from cases of diabetes with a view to discovering whether the glycosuria was associated with active degenerative changes in the pancreas or not.

---

[1] See *The Pancreas: Its Surgery and Pathology* (W. B. Saunders Co., 1907), p. 243.

[2] See Cammidge, *Proc. Roy. Soc.*, 1909.

In this modified method a specimen of the urine is boiled with hydrochloric acid, neutralised, treated with tri-basic lead acetate, and the excess of lead removed with sulphuretted hydrogen in the usual way. It is subsequently warmed to free it from sulphuretted hydrogen, mixed with yeast, and incubated to remove the fermentable sugar; but, as the " unfermentable " sugars are slowly broken down by the action of organisms contained in the yeast, it is necessary that the incubation should be stopped as soon as the last trace of fermentable sugar has been removed. This point is determined by simultaneously incubating a slightly larger quantity of a " control " specimen of urine, that has been dealt with in exactly the same way, except that it has *not been boiled* after the addition of the hydrochloric acid, and testing it at frequent intervals for sugar. Immediately no reaction is obtained the phenylhydrazin test is carried out with the specimen that has been hydrolised. The essence of the test therefore consists in fermenting *a control* only just so long as is necessary to remove the last trace of fermentable sugar, and taking this as a guide to the condition of the sample to be tested.

I have examined 296 specimens of urine from 168 cases of diabetes by this method, and obtained a positive result, suggesting that the diabetes was probably of pancreatic origin, in 121 (72 per cent.) of the patients. In 47 no reaction was obtained. Of the 47 whose urine gave no reaction two were children, and when the pancreas of one of these was examined post-mortem no abnormality could be discovered, either microscopically or macroscopically; the pancreas of the other was not examined after death. Two others in this group were also examined post-mortem and the pancreas was said to be normal. In three of the cases that gave a positive reaction the glycosuria followed an attack of acute, or subacute, pancreatitis from which the patient recovered. Twenty-six were believed to have had chronic pancreatitis, secondary in eight to the presence of gall-stones in the common bile duct, to an attack of typhoid fever in one, to chronic indigestion and duodenal catarrh in eighteen, to duodenal ulcer in three, associated with the presence of pancreatic calculi in one, and with a cyst of the pancreas in two. In one there was transient glycosuria associated with mumps, and one patient had had an accident involving the upper abdomen. Arterio-sclerosis was found in ten, two were syphilitic, and in seven there was a history of gout. In six there was primary malignant disease of the pancreas, and in two extension of a malignant growth from a neighbouring organ to the pancreas (cancer of the duodenum in one, and cancer of the common bile duct in the other). Eight of the cases were examined after death; two showed well-marked interacinar pancreatitis; in five there was

advanced interlobular pancreatitis, and in one well-marked fibrosis of the pancreas associated with calculi in the ducts.

At the best, however, the fermentation test is not altogether satisfactory. It is very laborious, requires constant attention, and is open to many fallacies. I have for long felt that if the " pancreatic " reaction could be made a quantitative one, and could be carried out by a method which would not be influenced by the presence of dextrose, &c., its findings would be of much greater value. After a lengthy series of experiments I have eventually evolved a procedure which appears to fulfil these requirements.

One hundred c.c. of the filtered urine are mixed with 5 c.c. of hydrochloric acid (sp. gr. 1·16), and boiled on a sand-bath for ten minutes. It is then cooled, made up to 100 c.c. with distilled water, and the excess of acid neutralised with 16 grams of lead carbonate. After standing for a few minutes it is carefully filtered, and the precipitate well washed with cold water, the washings being added to the filtrate. The filtrate is then thoroughly shaken with 12 grams of tribasic lead acetate, filtered, and the precipitate well washed with water. Several filtrations may be necessary at this stage. To the clear filtrate is now added 1 c.c. of ammonia (sp. gr. 0·880), and the precipitate that forms is collected on an asbestos filter. This precipitate is washed until the washings come through neutral, first with tap-water, and finally with distilled water. The precipitate is now dissolved in 11 c.c. of hydrochloric acid (sp. gr. 1·195), washed with water, and the mixed solution and washings made up to 50 c.c. The mixture is then submitted to steam distillation, and 50 c.c. of the distillate collected. This is neutralised with 20 per cent. caustic soda solution, using methyl orange as the indicator, and then made faintly acid with $\frac{N}{10}$ hydrochloric acid. Ten c.c. of an $\frac{N}{10}$ solution of sodium hydrogen sulphite solution are now added, and the mixture left to stand overnight. Next day 5 c.c. of $\frac{N}{10}$ iodine solution are introduced, and the excess of sodium hydrogen sulphite titrated with $\frac{N}{100}$ iodine solution, using two drops of starch paste as the indicator, until the blue colour persists for fifteen seconds. As a control the titration is repeated with 50 c.c. of distilled water to which have been added 10 c.c. of $\frac{N}{10}$ sodium hydrogen sulphite and 5 c.c. of $\frac{N}{10}$ iodine solution, until the same tint is obtained. The difference between the quantities of $\frac{N}{100}$ iodine solution used in the two experiments is termed " the percentage iodine coefficient " of the urine. This, multiplied by the total twenty-four hours' output of urine expressed in decilitres and decimals of a decilitre, gives the " total iodine coefficient."

I have found that the iodine coefficient of healthy urines is nil, and that even in those who are following sedentary occupations or have symptoms pointing to slight digestive or hepatic troubles, the percentage coefficient rarely exceeds 1 or 1·5. In cases of pancreatitis, on the other hand, it generally ranges from 12 to 20 per cent., with a total of 100 to 200 for the twenty-four hours. Similar figures have been obtained with several cases of malignant disease of the pancreas. A total daily coefficient of 100, or over, has been given by 75 per cent. of the cases of untreated diabetes that I have investigated, a result comparable with that obtained by the fermentation process. In a few cases of severe diabetes readings of between 400 and 500 have been obtained. The coefficient has been found to fall as a result of suitable treatment, ultimately reaching a total of only 5, or even 2·5, in some cases that responded well. In others it has not been possible to reduce it below about 50, and these have generally been cases in which no form of treatment was followed by permanently satisfactory results. That the iodine coefficient of the urine is independent of its sugar-content is shown by the results of numerous experiments in which various sugars have been added to normal urines. Dextrose, up to as much as 15 per cent., has given a negative result. With levulose it was also negative up to 2 per cent., and after that rose 1·2 for each 5 grams of the added sugar ; as, however, the levulose found in diabetic urines, unlike that of plant origin, is removed by treating the urine with basic lead acetate, it is not likely to introduce any serious error. With lactose and maltose a negative iodine coefficient was given by quantities up to 2 per cent. The coefficient for arabinose and xylose has been found to be about 30 to 35 for each 0·1 gram of the added pentose.

Adrenalin Mydriasis.—In 1907, Loewi stated that the instillation of adrenalin into the conjunctival sac has no appreciable effect on normal animals, but that after the pancreas has been removed rapid and pronounced dilatation of the pupil occurs. He suggested that this mydriatic effect might be of use as a sign of pancreatic insufficiency. On applying the test to the human subject, using a few drops of a 1 : 1000 solution of adrenalin or some similar suprarenal preparation, he found that a positive result was obtained with ten out of eighteen cases of diabetes, a marked dilatation of the pupil, that commenced in a few minutes and lasted for some time, taking place, but that only two out of thirty patients suffering from a variety of other diseases gave a reaction. Loewi's findings were confirmed in two cases by Glässner, and also in several cases by Schwarz, who, however, obtained negative results more often in

pancreatic diseases, and several times in ordinary diabetes and exophthalmic goitre, '' that is in morbid conditions, one of which certainly, and the other possibly, stand in some etiological relation to the pancreas.'' Quadrio examined twenty-five patients and found that twenty, who presented no evidence of pancreatic disease, gave no reaction, but that pronounced mydriasis resulted in five. One of these was an epileptic, and the other four had tumours of the pancreas or were suffering from advanced diabetes. The explanation of the reaction suggested is that the normal pancreas exerts an inhibitory effect on the sympathetic nervous system and prevents the action of adrenalin on it, so that when the gland is removed, or ceases to function, the sympathetic becomes more excitable and adrenalin exerts its full effect.

Although it is certain that the methods at present available are not capable of detecting even the majority of cases of glycosuria dependent upon lesions of the pancreas, it is possible by using one or more of the preceding tests, or better by employing several in conjunction, to demonstrate insufficiency of the gland with a considerable degree of certainty in a few instances. In others the results are indefinite or conflicting, so that, at the best, only a provisional diagnosis can be made.

BIBLIOGRAPHY

Acard, *Thésis*, Paris, 1895.
Adler, *Zeit. f. Heilkunde*, 1904.
Anschütz, *Deut. Arch. f. klin. Med.*, 1899.
Baldwin, quot. Herter, *Lect. on Clin. Path*, 1898.
Barker, *Med. Record*, 1908.
Barrenscheen, *Biochem. Zeit.*, 1912.
Baumgarten, *Zeit. f. exp. Path. u. Therap*, 1905.
Béclère, *Gaz. Med. d. Paris*, 1894.
Benda and Stadelmann, *Deut. med. Woch.*, 1896.
Bernard, *Leçons sur le diabète*.
Bernardt, *Sympt. u. Diag. d. Hungeschwülste*, 1865.
Bernoulli, *Corresp. Blatt. f. Schweizier Aerzte*, 1910.
Beyea, *Amer. Journ. of Obstet.*, 1900.
Bloch, quot. Oser, Nothnagel's *Ency. of Pract. Med.*, 1903.
Blumenthal, v. Noorden's *Clinical Medicine*, 1906.
Bönniger, *Deut. med. Woch.*, 1908.
Bosanquet, *Lancet*, 1905, 1909.
Borchardt, *Zeit. f. klin. Med.*, 1908.
Bouchardat, *Traité du Diabète*, 1875.
Brentano, *Arch. f. klin. Chir.*, 1900.
Bright, *Med. Assoc. Trans.*, 1833.

Brunton, *St. Barth. Hosp. Rep.*, 1874.

Burghart, *Verein. f. inn. Med.*, 1897.

Cammidge, *Lancet*, 1904, 1906 ; *Med. Chi. Trans.*, 1906 ; *Surg. Gynecol. and Obst.*, 1908 ; *Proc. Roy. Soc.*, 1909 ; *Proc. Roy. Soc. of Med.*, 1910 ; *The Pancreas, Its Surgery and Pathology*, 1907.

Cannon, *New York Med. Journ.*, 1912.

Cavazzani, *Zeigler's Centralb.*, 1893; *Centralb. f. inn. Med.*, 1894.

Cecil, *Journ. of Exp. Med.*, 1909.

Chauffard and Ravant, *Arch. d. Méd. exp. et d'Anat. path.*, 1901.

Cohnheim, *Zeit. f. phys. Chem.*, 1903.

Da Costa and Beardsley, *Amer. Journ. Med. Sci.*, 1907, 1908.

Cowley, *Lond. Med. Journ.*, 1788.

Croom, *Brit. Gynæcol. Journ.*, 1895.

Cushing, *Johns Hopk. Hosp. Bull.*, 1911.

Dallemange, *Arch. d. Méd. exp.*, 1895.

Decker, *Bull. State Univ.*, Iowa, 1908.

Dieckhoff, *Beiträge z. Path. Anat. Praxis.*, 1895.

Dompeling, *Nederl. Arch. v. Genel. Natuurk.*, 1868.

Dule, *Dublin Hosp. Gaz.*, 1857.

Dumontpelier, *Compt. rend. d. Soc. d. Biol.*, 1867.

Dutourier, *Thésis*, Paris, 1895.

Ehrlich and Frerichs, *Zeit. f. klin. Med.*, vi.

Ehrmann, *Arch. f. ges. Physiol.*, cxix. ; *Berl. klin. Woch.*, 1912.

Eichler and Silbergleit, *Berl. klin. Woch.*, 1908.

Eppinger, Falta, and Rudinger, *Wein. klin. Woch.*, 1907 ; *Zeit. f. klin. Med.*, 1908–9.

Erdheim and Stümme, *Beitr. z. path. Anat. u. z. allg. Path.*, 1909.

Ewald, *Berl. klin. Woch.*, 1895.

Falta, *Zeit. f. klin. Med.*, 1910.

Fedeh and Romanelli, *Riforma Medica*, 1909.

Ferrand, *Rev. Neurolog.*, 1901.

Fitz, *Med. Congress, Washington*, 1903.

Frerichs, *Ueber Diab. mellitus*, 1893 ; *Virchow's Arch.*, 1903.

Funck, *Münch. med. Woch.*, 1909.

Garrod, *Lancet*, 1912.

Gentes, *Thésis*, Bordeaux, 1901.

Goetsch, Cushing, and Jacobson, *Bull. Johns Hopk. Hosp.*, 1911.

Goiffon and Tallarico, *Arch. d. Malad. d. l'App. Digestif.*, 1912.

Goodman, *Annals of Surgery*, 1909 ; *Internat. Clinic*, 1909.

Grawitz, *Fortschr. d. Med.*, 1897.

Grimbert and Bernier, *Presse Médicale*, 1910.

Gross, *Deut. med. Woch.*, 1909.

Grossman, *Berl. klin. Woch.*, 1879.

Grübe, *Zeit. f. klin. Med.*, 1895.

Gründ, *Zeit. f. physiol. Chem.*, xxxv.

Guleke, *Arch. f. klin. Chir.*, lxxxvii.

Handmann, *Deut. Arch. f. klin. Med.*, 1911.

Hanot and Chauffard, *Rev. de Méd.*, 1882.

Hanseman, *Zeit. f. klin. Med.*, 1894 ; *Berl. klin. Woch.*, 1887, 1897 ; *Verhand. d. deut. path. Gesellsch.*, 1902 ; *Berl. klin. Woch.*, 1912.

Hanyn, *Der Diabetes.*

Hartmann, *Inaug. Dissert.*, Tübingen, 1878.

Heiberg, *Wien. klin. Woch.*, 1910 ; *Deut. Arch. f. klin. Med.*, 1911.

Henkel, *Deut. med. Woch.*, 1909.

Herlitzka, *Roy. Accad. d. med. d. Torino*, 1908 ; *Pflüger's Arch.*, 1908.

Herxheimer, *Virchow's Arch.*, 1906 ; *Verhand. d. deut. path. Gesellsch*, 1907.

Herzog, *Virchow's Arch.*, 1902.

Hirschfeld, *Berl. klin. Woch.*, 1905, 1908 ; *Deut. med. Woch.*, 1910.

Hürter, *Mediz. Klinik.*, 1910.

Imlach, *Brit. Med. Journ.*, 1885.

James, *Brit. Journ. of Dermatol.*, 1894.

Jeanselme, *Bull. d. hôp. d. Paris*, 1897.

Jodry, *Lehere Diab. Suisse*, 1909.

Jolles, *Wiener med. Woch.*, 1911.

De Jonge, *Arch. f. Psychiat.*, 1882.

Kehr, *Münch. med. Woch.*, lvi. ; *Mitteil a. d. Grenzgeb. d. med. u. Chir.*, xx.

Kinnicutt, *Med. Record*, 1909.

Klebs and Munk, *Hanb. d. path. Anat.*, iii.

Klemperer, *Verhand. d. Vereins f. inn. Med.*, 1896 ; *Berl. klin. Woch.*, 1892.

Koziczkowsky, *Zeit. f. klin. Med.*, 1909.

Krienitz, *Arch. f. Verdauungn. Krank.*, xv.

Lancereaux, *Semaine Méd.*, 1895.

Lannois and Roy, *Arch. gén. d. méd.*, 1903 ; *Semaine Méd.*, 1903.

Lefas, *Arch. gén. d. méd.*, 1900.

Lépine, *Rev. Scientifique*, 1891 ; *Rev. d. Méd.*, 1896 ; *Compt. rend. d. Soc. d. Biol.*, 1903 ; *Lyon Médicale*, 1903; *Journ. d. Phys. et d. Path. gén.*, 1905 ; *Diabète Sucré*, 1909.

Levrat and Perrotton, *Thésis*, Paris, 1859.

Levy, *Medical Record*, 1910.

Lewinski, *Deut. med. Woch.*, xxxiv.

Loeb, *Deut. Arch. f. klin. Med.*, 1884 ; *Centralb. f. inn. Med.*, 1898

Loewi, *Münch. med. Woch.*, 1907.

Lowei, *Arch. f. exp. Path. u. Pharm.*, 1902.

Lüthje, *Münch. med. Woch.*, 1901 ; *Cong. f. inn. Med.*, 1907.

Luys, *Gaz. Méd. d. Paris*, 1869.

Maass, *Mediz. Klinik.*, 1909.

Mannkopf, quot. Naunyn, *Die Diab. Mellit.*, 1906.

Marck, *Zeit. f. Gynæcol.*, 1911.

Margin, *Rev. d. Méd.*, 1905.

Marie, *Semaine Méd.*, 1895.

Marinesco, *Compt. rend. d. Soc. d. Biol.*, 1895 ; *Semaine Méd.*, 1895.

Mautry, *Brit. Med. Journ.*, 1889.

Mendel and Jackson, *Amer. Journ. of Physiol.*, 1902.

Michael, *Deut. Arch. f. klin. Med.*, 1889.

Minkowski, *Arch. f. exp. Path. u. Pharm.*, 1886, 1893, 1908.

Mirallié, *Gaz. d. hôp. d. Paris*, 1893.

Montgomery, *Journ. Amer. Med. Assoc.*, 1912.

Mosler, *Deut. Arch. f. klin. Med.*, xv.

Müller, *Verhand. d. xxiii. Cong. f. inn. Med.*, 1906 ; *Arch. f. klin. Med.*, 1908.

Nash, *Lancet*, 1901.

Naunyn, Nothnagel's *Spec. Path. u. Therap.*, 1898 ; *Der Diab. Mellitus*, 1906.

Neubauer, *Biochem. Zeit.*, 1905.

Von Noorden, *Diab. Mellitus*, 1906 ; *Die Zuckerkrank u. ihre Behand.*, 1910 ; *Mediz. Klinik.*, 1911.

Norris, *Proc. New York Path. Soc.*, 1907.

Ogle, *St. George's Hosp. Rep.*, 1866.

Opie, *Journ. Expt. Med.*, 1899, 1901 : *Diseases of the Pancreas*, 1903.

Oser, Nothnagel's *Ency. of Pract. Med.*, 1903.

Pavy, *Lancet*, 1908.

Perwuschin and Foworski, *Neurol. Centralb.*, 1900.

Pflüger, *Arch. f. ges. Physiol.*, 1907, 1908.

Pilcher, *Annals of Surgery*, 1910.

Pinches, *Jahrb. d. Wiener. Krank*, 1897 ; *Bull. d. Sci. Med. d. Scula Med. d. Bologna*, 1897.

Porges, *Wien. klin. Woch.*, 1909 ; *Zeit. f. klin. Med.*, 1910.

Porges and Salomon, quot. v. Noorden, *Die Zuckerkrank*, 1910.

Potter and Milne, *Amer. Journ. of Med. Sci.*, 1912.

Proescher, Grafe's *Arch. f. Ophthal.*, 1899.

Pusey, *Arch. f. Ophthal.*, 1904.

Quadrio, *Il Policlinico*, xv.

Quintard, *Bull. d. Soc. méd. d. Ang.*, 1894.

Rabé, *Bull. d. Soc. Anat. d. Paris*, 1900.

Ravaut, *Soc. méd. d. hôpit. d. Paris*, 1900.

Reale and Renzi, *Verhand. d. X. internat. Med. Cong.*, 1890 ; *Wien med. Woch.*, 1891 ; *Berl. klin. Woch.*, 1892.

Von Recklinghausen, *Tagebl. d. lxii. Versam. deut. Naturf. u. Aertze*, 1889.

Reichenstein, *Wien. klin. Woch.*, 1909.

Richardson, *Med. Times and Gaz.*, 1866.

Richartz, *Zentralb. f. inn. Med.*, 1910.

Rolleston, *Diseases of the Liver*, 1905.

Rosenbaum, *Arch. f. ges. Physiol.*, cxxi.

Rosenberger, *Zeit. f. Biol.*, 1907.

Rössle, *Vern. deut. path. Gesellsch.*, 1907.

Roth, *Zeit. f. klin. Med.*, 1909.

Sauerbeck, *Virchow's Arch.*, 1904.

Saundby, *Lect. on Diabetes*, 1891.

Schäfer, *Lancet*, 1895.

Schleisinger, *Wien. klin. Rundschau*, 1900 ; *Gesell. f. inn. Med.*, 1902.

Schmey, *Centralb. f. inn. Med.*, 1897.

Schmidt, *Prog. u. Therap. d. Zuckerkrank*, 1892 ; *Münch. med. Woch.*, 1902 ; *Mitteil a. d. Genz. d. Med. u. Chir.*, 1909.

Schroeder, *Journ. Amer. Med. Assoc.*, 1908.

Schwarz, *Wiener klin. Woch.*, 1909.

Scott, *Journ. of Path. and Bact.*, 1907.

Seegen, *Der Diab. Mellit.*, 1893.

Siebke, *Deut. med. Woch.*, 1910.

Silver and Irvine, *Trans. Path. Soc.*, 1878.

Smith, *Brit. Med. Journ.*, 1883.

Speese and Goodman, *Amer. Journ. Med. Sci.*, 1909.

Ssobolew, *Centralb. f. allg. path. u. path. Anat.*, 1900 ; *Arch. f. path. Anat.*, 1902.

Stadelmann, *Deut. Med. Woch.*, 1901 ; *Zeit. f. klin. Med.*, 1904.

Stiles and Lusk, *Amer. Journ. of Physiol.*, 1903 ; *Deut. Arch. f klin. Med.*, 1904.

Still, *Encyclop. Med.*, 1901.

Strümpell, *Deut. Zeit. f. Nervenheilk.*, 1897.

Sweet, *Journ. Med. Res.*, 1903.

Tachau, *Deut. Arch. f. klin. Med.*, 1912.

Trevor, *Practitioner*, 1904.

Turner and Stewart. *Text-book of Nervous Dis.*, 1910.

Volhard, *Münch. med. Woch.*, 1907.

Watson, *Lancet*, 1908 ; *Brit. Med. Journ.*, 1908.

Weichselbaum, *Wiener. klin. Woch.*, 1911.

Weiland, *Deut. Arch. f. klin. Med.*, 1911.

West, *Path. Soc. Trans.*, 1890.

White, *Path. Soc. Trans.*, xxxvi.

Wilks, *Lancet*, 1875.

Williamson, *Diabetes Mellitus*, 1898.

Windle, *Dublin Journ. Med. Sci.*, 1883.

Woolley and Newburgh, *Journ. Amer. Med. Assoc.*, 1911.

Zack, *Wiener klin. Woch.*, 1908.

CHAPTER VIII

BEFORE proceeding to deal with the metabolism in diabetes, it is advisable that we should briefly consider the metabolism and food supply of the healthy organism.

By metabolism in its widest sense is meant the sum of the chemical changes that go on in the body, but it is also used in a restricted sense to mean the processes by which the cells incorporate food materials of various kinds into their substance. The metabolic changes that go on in the tissues are of two kinds—(1) constructive, or anabolic, involving change from a lower to a higher state; (2) destructive, or katabolic, in which the reverse occurs, with the formation of various waste products. The former are chiefly concerned in the maintenance of the tissues of the body, while the latter give rise to the phenomena of motion that constitute life, and are the source of the energy of the living organism. In early life the metabolic processes of the body are more intense than later, and anabolism outstrips katabolism, with the result that the body grows. In later life the two processes are more nearly balanced, and after growth has ceased they should be approximately equal if the individual is to remain healthy. For this condition to exist it is necessary (a) that the supply of food material reaching the tissues should be sufficient; (b) that it should be in a form that can be utilised; (c) that the physical condition of the environment should be suitable. With the last we are not at present concerned, but the other two are of great importance.

The questions that arise then are: What is a sufficient food supply, and how are we to ascertain that it is being made use of by the tissues? The answer to the latter is the more easily obtained, for by a regular use of the scales we can determine whether weight is being lost or gained—that is to say, whether katabolism or anabolism preponderates. The information obtained in this way will partly answer the first question, but not completely, since a deficiency may not be in bulk but kind, and a loss of weight may be due to the food not being of a nature, and in the proportions that are most suitable. The direction and nature of the metabolic processes going on within the body can be determined in another

way—viz. by balancing the intake against the output. In the intake are included the food and oxygen, in the output the urine, the fæces, &c. To make a complete quantitative analysis of the ingesta and excreta would involve an enormous expenditure of time and labour, and as a rule sufficient information can be obtained by considering two elements only, the nitrogen and the carbon. Of these the nitrogen is the more important, for the nitrogenous foods are the only elements of the diet able to repair tissue waste, and the nitrogen content of the excreta is a measure of the tissue destruction that is going on within the body. When the nitrogen of the food exactly balances the nitrogen excreted, the body is said to be in " nitrogenous equilibrium," and it may be assumed that the living material of the tissues is not being increased or diminished in amount. For practical work in dietetics a suffi- ciently reliable estimate of the nitrogen content of the food is obtained by weighing it, working out the amount of protein it contains, and allowing 1 gram of nitrogen for each 6·25 grams of protein, since meat protein contains an average of about 16 per cent. of nitrogen. It is usually not necessary to estimate the nitrogen contained in the fæces, for, as a rule, only a small fraction, about 1 or 2 grams a day, escape absorption and are lost in the stool ; it is therefore only needful to determine the nitrogen content of the urine. This is estimated by Kjeldahl's process, or some modifica- tion of it.

A different level of nitrogenous equilibrium exists in different individuals, and it may also vary in the same person at different times. If the amount of nitrogenous food is diminished the amount of urinary nitrogen will also decrease. Should the amount of food then remain constant the output of nitrogen will likewise remain the same ; but if, on the other hand, more nitrogen is ingested an increased elimination will result, and at the same time a certain proportion will be retained by the body. so that gradually a higher level of equilibrium is established. There is a natural limit, however, to this power of accommodation, and a point is finally reached, varying with different individuals, where a further increase in the amount of ingested nitrogen does not lead to a higher level of equilibrium, and where, consequently, a further retention of nitrogen does not occur. Over-feeding then results and various digestive disturbances, such as diarrhœa and vomiting, follow. If a person in nitrogenous equilibrium takes an insufficient amount of food it will lead to an increased destruction of the organised albumens, and more nitrogen will be lost in the urine than is ab- sorbed from the food. For a time, the reserve of fats and carbo- hydrates is capable of protecting the body against an unduly rapid

loss of nitrogen, but finally the protection fails and death occurs from inanition.

It does not follow that because the body is in nitrogenous equilibrium that the other elements of the food and excreta are also balanced, and we may have an increase in the fats, and to a less extent in the carbohydrates, or the reverse, shown by the carbon, hydrogen, and oxygen of the ingesta being more. or less, than in the excreta.

Every process of the body is attended with manifestations of energy, shown as mechanical work, electric currents, or heat. This energy is derived from the food, and is an expression of the potential energy that it contains. Now, the researches of Mayer and Joule showed that the amount of power, or energy, that can be obtained from a given weight of matter is connected with, and is proportional to, the heat given out during its combustion, and as heat is the simplest measure of potential energy that can be obtained, it is convenient to calculate both the potential energy of the food, and the work done by the organism, in terms of heat units. The standard measure of heat, or heat unit, is the *calorie*. This is the amount of energy required to raise 1 gram of water 1° C., and is equivalent to 425·5 units of work, or gram-metres— that is to say, the energy required to raise 1 gram of water 1° C. would lift a weight of 425·5 grams to a height of 1 metre.[1] It is, however, an advantage in practice to use a larger unit than this, viz., the amount of energy required to raise 1000 grams of water 1° C., which is termed the *Kilo-, or large, Calorie.*

Foods which on oxidation give out the greatest amount of heat should theoretically have the greatest capacity for producing work, but the heat equivalents of organic substances cannot be calculated from their chemical composition ; for part of the heat, varying with different substances, is used in the process of dissociating the molecules, &c., and the heat equivalent has therefore to be determined by direct calorimetric methods. Rubner, as the result of his experiments, came to the conclusion that the heat value of 1 gram of protein in an average diet is 4·1 calories, although slight differences exist between different forms (*e.g.* casein = 4·4, meat protein = 4·233, vegetable protein = 3·96 calories). For fats Stohmann's figures were—olive-oil = 9·384 cal., animal fat = 9·372, butter fat = 9·179 cal. Rubner therefore adopted 9·3 as the average heat value of 1 gram of fat in a mixed diet. The following heat values have been found for carbohydrates—dextrose, 3·692 to 3·755 cal. ; milk-sugar, 3·877 cal. ; cane-sugar, 3·959 to 4·001 cal. ; starch, 4·116 cal. Taking into account the predominating

---

[1] To convert kilogram metres into food pounds × 7·233.

importance of starch in the average diet, Rubner gave the carbo-
hydrate group a heat value of 4·1 calories. Rubner's "standard
values" have been widely adopted, and are generally used in
determining the heat value of a mixed diet. They are :—

1 gram of protein	4·1 calories
1 ,, ,, fat	9·3 ,,
1 ,, ,, carbohydrates	4·1 ,,

Atwater and Bryant, as the result of over four hundred experi-
ments, obtained slightly different results, which they consider are
the heat values absolutely available in computing the average
diet :—

1 gram of protein	4·0 calories
1 ,, ,, fat	8·9 ,,
1 ,, ,, carbohydrate	4·0 ,,

The difference between the two standards is probably to be
explained by the fact that Rubner used comparatively pure foods.
while the waste in the fæces in Atwater and Bryant's experiments
reduced the amount of available nutriment. Since the combustion
of 1 gram of fat produces 9·3 calories, and the conversion of 1 gram
of protein into urea, carbon dioxide, and water, and of 1 gram of
carbohydrate into carbon dioxide, each produces 4·1 calories, the
combustion of 100 grams of fat will give rise to an amount of energy
equal to that produced by 227 grams of protein, or carbohydrate.
This amount of protein, or carbohydrate, is said to be of the same
" isodynamic value " as 100 grams of fat.

An average adult man expends daily about 34 to 35 calories for
each kilogram of his body-weight when taking moderate exercise.
when at rest about 10 to 15 per cent. less, and when taking active
exercise about 10 to 15 per cent. more, so that a man weigh-
ing 70 kilos (154 lb.) doing light work would require about 2500
calories in the twenty-four hours. This amount of energy would
be supplied by—

118 grams of protein	484 calories
56 ,, ,, fat	521 ,,
500 ,, ,, carbohydrates . . .	2050 ,,
	3055 calories (Voit)

provided that all the food materials were fully utilised for the
purpose of heat production in the body. As a matter of fact, the
heat value of foods to the body is a little less than is shown by the
calorimeter, owing to the loss of unoxidised products in the ex-
cretions, &c. Atwater, in his more recent experiments, estimates
that 4½ per cent. of the nourishment taken is unutilised by the

organism, while Rubner gives a slightly higher figure, 5 to $5\frac{1}{2}$ per cent. A certain proportion of the food ingested is rendered value-less to the body as the result of putrefactive and fermentative changes in the stomach and intestine, or is lost in the fæces, so that the actual caloric value of the food material to the body is always less than the theoretical value of that taken by the mouth. As a rule, probably somewhere from 10 to 25 per cent. should be deducted from the calculated value to represent this loss, but in cases where abnormal putrefactive and fermentative changes are going on in the gastro-intestinal tract, a much larger deduction must be made.

The proportions of protein, fats, and carbohydrates in a mixed diet may be varied within limits, but generally it is found that one part of protein food is taken to each four or five parts of non-protein, and that of the latter one part of fat is taken to five or ten of carbohydrate. According to Voit, one-third of the protein should be animal and two-thirds vegetable. Wide variations, however, exist among different races, and among different social grades of the same race.

Protein Requirement.—In the distribution of the diet men-tioned above, and suggested by Voit for an average labourer working eight to ten hours daily, it will be noticed that 118 grams of protein a day are allowed. This allowance, which represents from 1·4 to 1·7 grams per kilogram of body-weight, was arrived at by a statistical method, and showed what the average labourer is in the habit of consuming. Rubner obtained a higher figure, 127 grams, for the same class, and Atwater allows 125 grams. For men doing hard work Voit gives 145 grams, Rubner 165 grams, and Atwater 150 grams.

Experiment has proved that with an ordinary diet on Voit's standard, muscular exertion increases the metabolism from 1·1 per cent. to 18·0 per cent., with an average of 15 per cent. The addition of 10 to 15 grams of protein daily to the physiological minimum, raising it to 44 or 45 grams, might therefore be expected to suffice for the protein requirement when an average amount of work is being performed. Siven found that a man weighing 65 kilos could be maintained in nitrogenous equilibrium for a short period on a diet containing as little as 4 or 5 grams of nitrogen, that is to say, on 25 to 31 grams of protein a day, but Munk and Rosenheim showed that dogs given a quantity of protein only sufficient to maintain nitrogenous equilibrium gradually lost strength, and suffered from digestive disturbances. Numerous investigations undertaken by Voit, Moleschott, Ranke, Foster, Munk, and others have shown that when no food is taken for twenty-four hours the amount of nitrogen

excreted varies from 7·5 to 12 grams, the variation probably being due to a difference in the amount of stored and circulating protein and glycogen in the organism. But if the starvation is continued the stored protein and glycogen is all consumed in a few days, and after a period of about five days all the nitrogen in the excretions will be derived from the disintegration of tissue proteins. In fasting people the amount of nitrogen excreted by the kidneys from the fifth day of starvation averages about 4·5 grams per day. If we allow a margin of 1 gram for other modes of excretion, we arrive at the conclusion that the amount of protein absolutely essential to prevent the destruction of tissue proteins is (nitrogen $5·5 \times 6·25 =$) 34 grams daily, or about 0·5 grams of protein per kilo of body-weight.

Chittenden has claimed that well-nourished men, following various vocations, can be maintained in good health for a period of several months on a mixed diet containing 40 to 60 grams of protein. He considers that the Voit standard contains about twice the necessary amount, and that the excess, consumed only from habit and self-indulgence, throws an unnecessary strain on the liver, kidneys, and other organs concerned in the transformation and elimination of the end-products of protein metabolism. Chittenden's results have been much criticised on the ground that the men tested were all of the better classes, and could undergo a greater restriction than the poorer classes, who are not so well nourished, also that the subsequent health of the cases was not reported, so that it remains to be seen whether the quantity of protein in his ration, which is not greater than would be metabolised in starvation, is advisable as a permanent standard. It cannot be denied, however, that 50 grams of protein, containing 8 grams of nitrogen, are apparently sufficient to maintain the machinery of the body in good repair. Voit himself has stated that a vegetarian can live in nitrogenous equilibrium on a diet containing 48·5 grams of protein, and that an active man, weighing 74 kilos, may remain in good condition on less than 118 grams.

It is believed that proteins undergo different metabolic changes according to the purpose they are to serve in the body, a part, the so-called " repair-proteins," appear to enter into the living substance of the tissue cells to make good the waste of their substance that the vital processes involve, while another part, the so-called " energy-protein," is used as a source of work and heat. The repair protein is slowly broken down, giving rise to the uric acid, creatinin, and neutral sulphur compounds of the urine. The energy protein appears to undergo a rapid process of denitrification, the nitrogen-containing fraction which is split off being oxidised and excreted

in the form of urea and inorganic sulphates, while the carbonaceous portion is used, like the carbohydrates and fat of the food, for the production of energy. The proportion of the total intake of protein used for energy production depends upon circumstances. If the food contains much more protein than is required to maintain nitrogenous equilibrium, the larger part is made use of in this way, but if only a small amount of protein is taken, the greater part, or even the whole, is used for tissue repair. The cleavage of protein is increased by muscular work, but the cleavage is small in proportion to the consumption of non-protein material, and it is not directly connected with the work performed. When the body contains no stored proteins muscular exertion increases the output of nitrogen 15 per cent. ; but if the body is in good " condition," that is, containing a store of circulating proteins, the output of nitrogen is larger, because such proteins are consumed as a source of heat and energy ; but the muscle fibres are not broken down in a greater proportion by the work they perform. In Paton's investigation the excretion of nitrogen was increased both during and after the performance of work, and the metabolism of protein, indicated by the increased nitrogen excretion, accounted for 35 per cent. of the work done. It was evident, therefore, that one-third of the energy expended in work was derived from the metabolism of nitrogenous, and two-thirds from non-nitrogenous matter.

It is not a matter of indifference, however, to the organism as to whether its energy is derived from a nitrogenous or a non-nitrogenous source, for not only are the waste products from the former more highly organised and more difficult to get rid of than from the latter ; but according to Rubner 28·6 per cent. of the energy of meat protein is never utilised in the life processes of the tissues, but is liberated as free heat during the early cleavage, while with cane-sugar, for instance, only 3·1 per cent. of its energy is dissipated as heat when it is inverted into levulose and dextrose. Rubner found that if the quantity of protein in the diet of a dog is raised above the requirement by 56 per cent., there is an increase in heat production of 19 per cent., with a rise of 90 per cent. heat production is increased 35 per cent., and with a rise of 105 per cent., 44 per cent. more heat is produced. The temperature scarcely changes so perfect is the regulatory mechanism, but more rapid respiration indicates the increased oxidation and the efforts of the body to rid itself of the excess of heat. This effect of abundant protein food in raising metabolism is called by Rubner the " specific dynamic " action of protein.

In a state of health, the nature and amount of the other constituents of the diet exert an influence on the direction of protein

metabolism. The presence of carbohydrates, fats, and gelatine in the food enables a larger proportion to take part in tissue repair, shielding it from denitrification and from being used for purposes of energy production. Such substances are known as " protein sparers." The exact mechanism of their action is not understood, but it is possibly the effect of " mass influence." If such is the case, it is obviously important that a low protein ration should be spread as evenly as possible over the day, and be thoroughly blended with the non-nitrogenous food materials. The intimate mixture of protein and carbohydrates that exists in vegetable foods possibly explains why nitrogenous equilibrium is attained more readily on a vegetable than on any other form of diet. It would also seem that the kind of protein is not altogether a matter of indifference, but that some varieties form amino acids that more readily yield their carbohydrate moiety than others.

Fats and Carbohydrates.—The fats and carbohydrates are the chief source of energy, and are much more economical, both financially and physiologically, than proteins. To obtain equivalent amounts of energy, for instance, from protein and fat eleven and a half times more of the former by weight must be destroyed than of the latter, since each gram of nitrogen that is lost corresponds to a diminution of body-weight of 33 grams by " flesh," with an energy yield of 0·8 calories per gram, while the oxidation of 1 gram of fat simply means the loss of 1 gram of body-weight with an energy yield of 9·3 calories. The ingestion of carbohydrates or fat alone, while supplying power to the organism, does not prevent tissue waste, and, although death is delayed by a fatty or carbohydrate diet, it eventually ensues from the gradual weakening of the vital organs that takes place.

Fat.—Voit showed that the ingestion of 100, 200, and 300 grams of fat by a fasting animal scarcely influences protein metabolism at all, tending, in fact, rather to increase than to diminish it if anything. It would seem that the ingested fat is simply burned in place of the body fat, the total consumption of protein and fat remaining unchanged. Schulz found that during starvation there is an increase in the quantity of fat in the blood, and Rosenfeld showed that the quantity of fat in the liver rises to 10 per cent. during fasting, and to as much as 25 per cent. if fats alone are taken.

If carbohydrates are given to a fasting animal the fat-content of the liver falls to 6 per cent., but if carbohydrates are given along with fats the latter are not retained by the liver as they are when taken alone, so that there appears to be an antagonism between glycogen and fat deposition in the liver. The effects on metabolism

T

produced by the ingestion of fats and protein together have been investigated by Voit and Korkunoff. They found that much less protein food is required to maintain nitrogenous equilibrium when the two are taken together than when proteins are consumed alone. and that with increasing quantities of fat there is, for a time, an increased addition of protein to the body. Eventually, however, a higher level of nitrogenous equilibrium is established and the deposition of protein ceases, any excess being used for energy production. as on a protein diet.

The continued administration of an excess of fat to the healthy organism leads to its being stored. first in the subcutaneous and intermuscular connective tissue, and then in the abdominal cavity. In the converse condition, when energy is required to meet a chronic deficit in the intake, the abdominal fat is made use of first, then the subcutaneous fat. after that the intermuscular deposits, while the fat in the organs is only used as a last resort.

Little is known of the stages through which fat passes in the course of its utilisation by the body, probably the first step is similar to that which it undergoes in digestion, a cleavage into glycerine and fatty acids. That beta-oxybutyric acid is one of the products of the metabolism of fats is suggested by a study of the chemical pathology of diabetes, but Satta and others consider that the available evidence is against this substance being a normal cleavage product. The heat production in the early cleavage processes of fat appears to be small, and Rubner calculated that the specific dynamic action of fat raises the metabolism 14·4 per cent. at a temperature of 31° C. Allowing for the difference in the specific dynamic action of protein and fat, it would appear that these two substances can each replace the other in metabolism in isodynamic proportions.

Carbohydrates.—This class of foodstuffs has been found to protect the tissues from wasting more effectually than fat. Voit gave a fasting dog 500 grams of sugar and noticed a fall in protein metabolism from 181 to 170 grams, and Rubner was able to reduce the nitrogen in the urine in a fasting man from 11·9 to 6·3 grams by giving carbohydrates.

The specific dynamic action of carbohydrates is low, the heat produced in the early cleavage processes being smaller than for fats. It has been calculated by Rubner that cane-sugar, for example, raises the metabolism 5·36 per cent. on an average. Hence the ingestion of sugar by a starving animal raises the general metabolism to little above the starvation requirement, the small specific dynamic action of the sugar absorbed scarcely exceeding the reduction due to the diminution in protein loss, with its much greater

specific dynamic action, that ensues. This is well illustrated by an experiment of Rubner's, in which he compared the metabolism in starvation, on cane-sugar, and on meat :—

Starvation	2042	Calories
Sugar (120 per cent. requirement) . .	2087	,,
Meat (120 ,, ,,) . .	2566	,,

The administration of an easily absorbable carbohydrate not only reduces protein metabolism, but when given above the energy requirement it is retained within the body, part being stored as glycogen and part as fat. The conversion of sugar into glycogen is universally acknowledged, and definite proof of the conversion of carbohydrate into fat, which was doubted by some, was furnished by the experiments of Meissel and Strohmer, of Lehmann and Voit, of Rubner and others. When a substance rich in oxygen, like dextrose, is converted into material poor in oxygen, like fat, the intramolecular oxygen becomes available for purposes of oxidation, with the result that the volume of carbon dioxide expired may increase and be greater than the volume of the inspired oxygen. Johansson, Billstrom, and Heyl found that if 50 to 200 grams of cane-sugar were given to a fasting man the carbon dioxide output increased from 22·6 grams per half-hour to about 30 grams. More carbon dioxide was not found to be eliminated with large than with small doses, showing the regularity with which the process of utilisation proceeds, and indicating that any excess is stored until required by the organism. It has been shown by Sieven that the level of nitrogenous equilibrium can be lowered very considerably by giving carbohydrates along with protein, thus proving their marked action as protein sparers. This action has also been demonstrated in the reverse way by Lusk, who found that the sudden withdrawal of carbohydrates from the diet causes a rise in nitrogen metabolism. When, however, the carbohydrate in the diet is partly replaced by fat, protein metabolism is not influenced, or there is only a transitory change. Landergren proved that a diet containing half its calories in carbohydrates, and half in fat, has about the same protein protecting power as one made up of carbohydrate alone. If the carbohydrates of the food are entirely replaced by fat, protein metabolism rises, but is again reduced on the reintroduction of sugars and starches. The substitution of a carbohydrate-free for a mixed diet also raises the amount of acetone in the urine considerably above the average normal level, but it diminishes, or disappears again, when carbohydrates are taken. The fact that carbohydrate inanition is the sole cause of acetonuria is explained by Geelmuyden on the hypothesis that carbohydrates, or a derivative, glucuronic acid, unite with acetone

in intermediary metabolism, and that this process is necessary for the further change of the acetone bodies. If the synthesis is limited, or fails, a collection of the acetone bodies takes place, and they are consequently eliminated in increased quantities in the urine. To summarise the chief points in carbohydrate and fat metabolism, therefore, it would appear that the former are the most economical foodstuffs from every point of view ; they are the greatest sparers of protein, they may almost completely replace fat in the food, they are more completely absorbed from the intestine than fat when given in the form of sugar or cooked starch, although when contained in some vegetables the carbohydrate content of which is small relative to their bulk (*e.g.* spinach, lettuce, cabbage, &c.), a considerable proportion may escape absorption. After being absorbed they much more quickly oxidised than other food materials, and are therefore desirable when a quick supply of heat or energy is required. Further, they can be given in a greater variety of form than fat, so that appetite, which, after all, is a most important factor in dietetics, is not blunted by a lack of change. Fats, on the other hand, although they are a much more concentrated food, are more costly, both to the pocket and to the organism, an excess raising protein metabolism and giving rise to acetonuria when the use of carbohydrates is entirely abandoned.

Normally, the ingestion of fat has for its object the relief of the intestine from excessive carbohydrate digestion and absorption, but when a large amount is given for a protracted period digestive disturbances are apt to ensue. As fat is usually only given in five forms (butter, cream, cheese, and animal, or vegetable, fat), and there is a strong repugnance on the part of many individuals to fatty foods in any form, considerable skill is required to obtain variety, and to make it palatable, when more than the average amount is included in the diet. Experiments by Zuntz and Heineman have shown that there is very little difference in the efficiency of the body as a machine whether carbohydrates or fats are taken. Zuntz, for instance, found that in one experiment each kilo-gram-metre of work was accompanied by the liberation of 9·39 calories on a fat diet, and 10·37 calories with carbohydrate food. It is a matter of common experience, however, that muscular fatigue is delayed and more work is done when carbohydrates are available. The marked liability of diabetics to muscular fatigue also points in the same direction. Experimental confirmation of the intimate relation between a deficiency of carbohydrate and fatigue in voluntary muscle is supplied by the observations of Lee and Harrold, who found that the muscles of a cat, from which the readily combustible sugar had been swept out by treatment with

phlorhidzin, contracted only 200 to 400 times a minute on elec-trical stimulation, instead of 800 to 1000, as they should have done. Moreover, the curves of contraction resembled those of normal muscle in the late stages of fatigue. The muscles of control animals, which had been given phlorhidzin for four days and then re-ceived 50 grams of dextrose, were examined after an interval of eight hours, and it was found that their muscles gave 650 contrac-tions a minute, and that the first hundred were quite normal in character, thus showing that the results of the first experiment were not due to any poisonous action of the drug.

Alcohol.—The nutritive value of alcohol has been the subject of much discussion. According to Atwater and Benedict, small quantities can be used in the economy in the place of isodynamic quantities of carbohydrate or fat, but large doses, according to Miura, increase the waste of tissue protein. One gram of alcohol has a heat value of 7 calories, so that 30 to 60 grams (1 to 2 oz.) in the form of whisky, or Rhine wine, yield 270 to 540 calories, and a litre of German beer, containing 3 to 4 per cent. of alcohol and 5 to 6 per cent. of extractives, yields 450 calories, only half of which comes from the alcohol, however, the remainder being derived from the dextrin and protein-like extractives.

Alcoholic beverages are generally taken not for their food value, but as stimulants, and for the sake of their flavour. As a stomachic, alcohol is of little use when gastric digestion is normal; but it may be of service when the secretory powers of the stomach are de-fective, or to stimulate appetite, especially when considerable quantities of fatty food have to be consumed.

Metabolic Changes in Chronic Glycosuria.—A diabetic patient is in much the same position as a person with an insufficient food supply, for although the food is there his tissues are unable to make use of it in the normal way.

In mild cases, where the sugar disappears from the urine when carbohydrates are cut out of the diet, and where the patient is still able to make use of protein sugar, the metabolism of proteins is not different from that of a person living on a diet of meat and fat. Lusk has shown that if the conditions under which a diabetic lives be imitated in a healthy man by diminishing the carbohydrates of his food by an amount equivalent to the sugar excreted in the urine of the patient with whom he is being compared, protein destruction in the healthy individual is the same as in the diabetic.

In severe cases the conditions are more complicated. While in the healthy organism, and in mild diabetes, the supply of car-bohydrate derived from protein metabolism can be made use of

to protect the tissues from further destruction, in the severe type this is not the case, for when the protein sugar is withdrawn from the tissue cells there is a large increase in protein metabolism, and consequently in the nitrogen output in the urine. Thus in a case reported by Mendel and Lusk, it was found that the ingestion of broths containing 7·7 grams of nitrogen was followed by an elimination of 21·7 grams of nitrogen in the urine, or a loss of body nitrogen of approximately 14 grams, and that nitrogen equilibrium could only be maintained by giving 27 grams of protein nitrogen in the food. The abnormal tissue waste that occurs in such cases is commonly, although without definite proof, attributed to the action of toxines, and is consequently termed " toxogenic proteid disintegration."

Another result of the abnormal tissue destruction is the appearance in the urine of an excessive quantity of purin bodies, which are derived from the nuclear substance (endogenous purin bodies). In some cases an increase of 50 per cent., or even 100 per cent., has been met with, 0·25 to 0·30 grams of purin nitrogen being excreted in the urine daily. (Von Noorden.)

Proteins.—A large amount of information is now available with regard to the relation between the urinary nitrogen, and the sugar elimination, in the fasting and meat-fed diabetic organism. Since protein contains about 16 per cent. of nitrogen and 50 to 55 per cent. of carbon, 100 grams of protein material could theoretically give rise to about 130 grams of sugar and 16 grams of nitrogen, so that if both were completely eliminated in the urine a dextrose to nitrogen ratio $(D : N)$ of about 8 : 1 would be obtained. According to the observation of Rubner, however, 1 gram of protein nitrogen supplies 18·6 available calories, and, since 1 gram of dextrose furnishes 3·74 available calories, 4·97 grams of dextrose will be required to yield the same amount of energy. On this finding we should expect that at the most each gram of nitrogen in the urine can be accompanied by a little less than 5 grams of dextrose when the power to utilise sugar is completely lost. The experiments of Minkowski and others have shown that in depancreatised dogs on a protein diet, 2·8 grams of dextrose are excreted in the urine for each gram of nitrogen eliminated, and the same dextrose to nitrogen ratio has been found in the urines of a variety of animals rendered diabetic with phlorhidzin, or by removal of the pancreas. Assuming that these animals were incapable of using sugar, and that the ratio $D : N :: 2·8 : 1$ represents the amount of sugar derived from the cleavage of protein within the body, it would appear that rather less than 45 per cent. of the protein molecule is

converted into dextrose during metabolism. This follows from the fact that 1 gram of nitrogen in the urine corresponds to a destruction of 6·25 grams of protein in the organism, hence :—

$$1 \text{ gram nitrogen} = 6 \cdot 25 \text{ grams protein} = 2 \cdot 8 \text{ grams dextrose}$$
$$\therefore \frac{2 \cdot 8 \times 100}{6 \cdot 25} = 44 \cdot 8 \text{ per cent.}$$

It has been shown by Lüthje, however, that if depancreatised dogs fast completely after the operation, the excretion of dextrose in the urine ceases, while the percentage of sugar in the blood returns to the normal ; hence the bodies of these animals appear to be capable of utilising a limited amount of sugar. Reilly, Nolan, and Lusk, working with dogs made diabetic with phlorhidzin, discovered a higher dextrose to nitrogen ratio than was met with by Minkowski, $D : N :: 3 \cdot 65 : 1$, that would show a yield of slightly over 58 per cent. of sugar from protein :—

$$\frac{3 \cdot 65 \times 100}{6 \cdot 25} = 58 \cdot 4 \text{ per cent.}$$

This higher dextrose to nitrogen ratio, $3 \cdot 65 : 1$, was also found in some severe cases of diabetes when the patient was given a diet of meat and fat. They proved that in such cases the relationship between the nitrogen and sugar of the urine is constant, no matter how much protein is given, and is in no way dependent upon variations in the amount of fat in the diet.

The reason for the existence of the two ratios $2 \cdot 8 : 1$ and $3 \cdot 65 : 1$, each occurring when there is complete intolerance for carbohydrates, is not clear. Mendel and Lusk suggest that the sugar in the blood exists in two forms combined with colloid material. The one, a-colloid dextrose, corresponds to the amount of sugar represented by the lower ratio, or 45 per cent. of the protein ; while the other, β-colloid dextrose, represents the additional 13·6 per cent. of the protein when the higher ratio is present. The dextrose to nitrogen ratio found would then depend upon whether the β-dextrose were utilised or not. It is also possible that sugar production varies under different circumstances, and that the organism may be able to form sugar from a certain class of protein decomposition products at times only, and under certain conditions, when the higher ratio will be found.

Reilly, Nolan, and Lusk attempted to answer the question as to when protein sugar becomes available for use in the organism, by giving a fasting phlorhidzinised dog 500 grams of meat and collecting the urine at regular intervals. They found that the fasting relation between the dextrose and nitrogen changed immediately on the ingestion of the meat, more sugar being eliminated than corresponded to the nitrogen in the urine in the early hours, and less

in the later periods. It was therefore evident that the sugar elimination took place decidedly before that of the nitrogen, a fact that should be borne in mind when collecting the urine of diabetics for analysis. To allow of the complete elimination of the nitrogen corresponding to the sugar of a twenty-four hours' sample, it is advisable that the collection should terminate at an early morning hour, before any food has been taken.

The energy value of protein to the diabetic organism varies, of course, with the proportion of protein sugar that is excreted un-utilised in the urine. Taking an extreme case, where a dextrose to nitrogen ratio of $3·65 : 1$ exists, we have seen that $52·5$ per cent. of the available energy is lost as dextrose in the urine ; we have also seen that, according to Rubner, $28·5$ per cent. of the energy of meat protein is liberated as free heat that cannot be utilised in the life processes of the tissues, so that there only remains a balance of 19 per cent. that can be made use of for the vital processes of the body. To compensate for this great waste of energy there is a great increase in protein metabolism, hence in severe cases of diabetes 30, 40, or even 50 grams of nitrogen may appear in the urine in the twenty-four hours. The administration of a large amount of meat in severe cases of diabetes is liable not only to increase protein metabolism and the output of sugar, but also to interfere with the fixation of glycogen by the liver and thus tend to promote acidosis, so that a " carbohydrate-free " diet should never be given, at any rate for more than a few days, without very careful consideration.

Most observers are agreed that vegetable proteins are less harmful in severe cases of diabetes than those of animal origin, and that by substituting them in part, or completely, for meat in the diet the glycosuria is more readily controlled, and the danger of acidosis is more easily averted. Various preparations of vege-table protein are now on the market, and may be used for this purpose. The least expensive and most generally useful vegetable protein food is the soy bean (*Glycine hispida*). This contains $38·5$ per cent. of protein, and 20 per cent. of fat calculated on a water-free basis, and is almost starch-free, a phenomenon which is said to be due to the presence of a diastatic ferment capable of converting any starch formed into sugar (two-thirds) and dex-trine (one-third). It may be eaten as a vegetable, after being well soaked in water, as a salad, in soups, or the flour may be made into muffins, or cakes, &c., but it must be remembered that it contains from 20 to 40 per cent. of carbohydrate.

Fats.—It will be recalled that, though fats do not protect the tissues from wasting so effectually as carbohydrates, the healthy

organism can be maintained in nitrogenous equilibrium on a diet of meat and fat alone, and since the power to utilise fats is not apparently affected in the earlier stages of persistent glycosuria, fatty foods can be used in place of carbohydrates to meet the energy requirements of the body, and spare the proteins from excessive waste. As the condition progresses, however, fat metabolism is more or less impaired, and we see increasing wasting, lipæmia, and acetonæmia. A limitation of the fat in the diet, and its partial replacement by carbohydrate, is then advisable, even though the glycosuria may be thereby increased. Schwarz states that the lower fatty acids increase the amount of acetone bodies more than the higher members of the series ; butter, which contains more or less butyric and other lower fatty acids, causes, therefore, more marked acetonuria than animal fats that contain stearin and palmitin. The action of oil depends upon the nature of the con-stituent fatty acids, those containing the oleic groups having the least effect. Joslin considers that Schwarz's results may have been due to lack of absorption, for he found that oleic acid may nearly double the acetonuria in a fasting man, while butyric acid had no effect on the output of acetone bodies.

According to v. Noorden, there is in most cases of diabetes an impairment of the power of the body to synthesise fats from carbo-hydrate, and it is partly to this that the wasting seen in severe cases is to be attributed. In some individuals, although sugar utilisation is defective, the synthesis of fats from carbohydrates is not interfered with, so that the tissues being richly bathed with sugar build up excessive quantities of fat, and obesity results. So long as the power to form fat from sugar is unaffected glycosuria does not occur, but eventually there is, as a rule, a gradual impair-ment of the synthetic process, and sugar is consequently excreted in the urine. At first it may appear only intermittently and when a large amount of carbohydrate is consumed, but later it is excreted regularly, and we then have the common form of " *diabetes in the obese.*"

A mixed diet of protein and fat will not ordinarily increase the amount of sugar in the urine in diabetes, but in some cases the quantity of sugar eliminated appears to be greater than can be accounted for by the destruction of the protein of the food and tissues, as measured by the nitrogen excretion : it would therefore seem probable that under these conditions sugar is being formed from fat. Cremer found that the administration of glycerine, one of the cleavage products of fat, will increase the output of sugar in the urine, but there is no direct evidence that the higher fatty acids can be converted into carbohydrates, although v. Noorden main-

tains that this must occur, since, in some cases of diabetes, he has
found a larger amount of sugar in the urine than would be accounted
for from other known sources, including glycerine. Lusk states
that sugar is not formed from fat in phlorhidzin diabetes in animals,
and that if such a formation occurs in diabetes in man, it must be
due to a qualitative alteration in the metabolism in rare and special
cases. He and Mendel investigated the metabolism of dogs with
phlorhidzin glycosuria, starving, and after meat ingestion, and
found that the latter doubled the protein metabolism and caused
a fall in fat metabolism, as it would do in a normal animal. The
dextrose to nitrogen ratio remained unchanged, showing that the
amount of sugar was not influenced by the quantity of fat made
use of.

Carbohydrates.—Defective carbohydrate metabolism is the essen-
tial feature of the diabetic state, and there is always a more
or less marked difficulty in dealing with dextrose and substances,
such as starch, that give rise to dextrose in the processes of diges-
tion. We have seen, however, that the structure and configura-
tion of a sugar determine whether it shall, or shall not, be attacked
and broken down by living cells, so that it would appear possible
that the tissues of the diabetic organism might be able to make use
of sugars of different composition, or space arrangement, to dextrose.
Minkowski found that although the livers of depancreatised dogs
could not form glycogen from dextrose, the administration of levu-
lose resulted in glycogen being deposited, and caused a reduction
in protein metabolism, also that after 100 to 200 grams (10 to 20
grams per kilo) of levulose had been given to a depancreatised dog
only half reappeared in the urine, and of this 90 per cent. was
excreted as dextrose, 10 per cent. at the most being passed as levu-
lose. Observations on diabetic patients by Bouchardat, Külz,
v. Noorden, and others, have shown that in mild cases of diabetes
levulose causes less sugar to appear in the urine than dextrose, and
that there is at the same time a rise in the respiratory quotient.
The power to utilise levulose possessed by such individuals is
limited, however, and is soon overtaxed, so that its continuous
administration after a time is followed by almost as marked glycos-
uria as would be produced by an equal amount of dextrose. In
most severe cases of diabetes a varying proportion of levulose is
found, along with the dextrose, in the urine, and in such cases the
administration of levulose causes almost as much sugar to appear
in the urine as when dextrose or starch is given. Mendel and
Lusk investigated the urine of a severe case of diabetes, and found
that when 100 grams were taken an increase in the sugar excretion,
corresponding to 80 per cent. of the ingested sugar, took place,

and that it had no effect on protein metabolism. The fact that diabetics can frequently utilise levulose, although the glycogen formed from it must later be converted into dextrose, has been used as an argument in favour of the view that it is not as sugar that the cells make use of carbohydrates, but as glycogen. Neubauer has shown, however, that the power to form glycogen from levulose, as opposed to dextrose, is not a characteristic of diabetes, but is also seen in phosphorus poisoning. Arguing from the known relation of alimentary levulosuria to diseases of the liver and Minkowski's experiments on animals, it has been suggested that a failure on the part of a diabetic to make use of levulose points to the condition being dependent upon, or associated with, hepatic disease.

Pentoses have been suggested as a substitute for dextrose in diabetes, since Cremer and others have shown that a pentose, such as rhamnose, can be utilised by animals and spare an isodynamic equivalent of fat. Lindemann and May found that a healthy man could make use of 90 grams of rhamnose, but when this was given to a diabetic, whose urine had previously been sugar-free, glycosuria was produced. Von Jaksch found that rhamnose, arabinose, and xylose all increased the glycosuria, tended to raise protein metabolism, and caused diarrhœa when administered to severe cases of diabetes.

It has long been known that diabetics appear to assimilate some forms of starch much better than others, but that there is a very considerable individual variation in this respect, so that a dietary that will suit one will prove unsatisfactory for another. It has also been found that better results are obtained when one source of carbohydrate is employed alone than when several are mixed together. These differences depend probably in part upon the ease with which they are digested and assimilated, those being tolerated best which take the longest to absorb, and consequently pass only slowly into the blood. Many of the green vegetables, such as cabbage, lettuce, spinach, celery, asparagus, cucumber, &c., and some fruits, such as melon, pineapple, strawberries, rhubarb, &c., contain under 5 per cent. of carbohydrate combined with a considerable amount of indigestible cellulose, and as, under ordinary circumstances, a portion even of this starch is not inverted and absorbed, restriction of the diet to such substances is, as Blum points out, almost equivalent to fasting, so far as the tissues are concerned. The way in which these vegetables are prepared for the table exerts some influence on the amount of carbohydrate absorbed, and serves to explain the fluctuations in the amount of sugar excreted after their use. The least harmful source of sugar in each particular case can only be learnt by trial and careful

analysis of the urine ; at the same time an attempt should be made
to discover if the sugar-yielding food can be more safely given at one
period of the twenty-four hours than another, for such often proves
to be the case.

In his report on the results of monotonous feeding of prisoners
Bär says that the continual serving of one kind of food, always in
the same way, causes loss of appetite, vomiting, flatulence, and
diarrhœa, or obstinate constipation. Such results are not infre-
quently met with in diabetics who are restricted to a diet consisting
almost entirely of meat, fat, and the so-called diabetic breads, &c.
It has also to be remembered that stringent restriction of the diet
usually leads to disobedience and evasion, which is more harmful
than a little extra licence. By allowing the patient as much
vegetable as possible, and particularly the coarser kinds containing
a low percentage of carbohydrate, we not only give a much greater
variety of food, and so ensure a more ready acquiescence in our
directions, but also furnish a vehicle by which a much larger quan-
tity of fatty food can be taken with comfort than would other-
wise be the case. The alkaline salts contained in vegetables and
fruits no doubt assist in the treatment of diabetes, for they aid
in the neutralisation of the acids formed as the result of a highly
protein diet and faulty metabolism.

Diets based upon the different effects of various starchy foods
have from time to time been introduced. Mossé has recommended
potatoes, as they seemed to diminish the sugar and polyuria.
Although, in some cases, this dietary appears to exert a beneficial
effect, partly owing to the causes mentioned, and particularly to
its richness in potash salts, the treatment as recommended by
Mossé is now rarely used. It may be mentioned here that a small
amount of potato, thinly sliced, and cooked by being plunged into
boiling fat, is often a useful addition to the diet of a diabetic, for
one small potato prepared in this way will fill a tureen, give a
pleasant change, and have a food value of about 2600 calories per
pound. The best known and most frequently used of these
special diets is the oatmeal " cure " introduced by v. Noorden.
This consists in the daily administration of 200 to 250 grams (6 to 8
oz.) of oatmeal, 200 to 300 grams (6 to 10 oz.) of fat, in the form
of butter, and 100 grams ($3\frac{1}{2}$ oz.) of protein, in the shape of three
or four eggs or 50 to 100 grams of a vegetable protein, prepared
in the form of soup or porridge, and given at frequent intervals,
with an occasional allowance of black coffee, or tea with lemon-
juice, wine, or cognac. The oatmeal is prepared by placing it in
three parts of water, slightly salted, and thoroughly cooking it for
at least six hours ; while still hot it is strained through a sieve.

The coarse covering of the kernel that remains is rejected, and the butter is then stirred into the hot porridge that has passed through. After three or four days of such a diet there succeed one or two days on which only green vegetables are allowed, then the oatmeal cure is resumed. The treatment is continued in a similar manner for several weeks. As a rule four or five courses are required. According to v. Noorden, it is advisable to precede the oatmeal treatment by a few days of restricted, or vegetable, diet, but Crofton states that in his experience this is unnecessary, and is in fact rather detrimental than otherwise. At the commencement of the cure the glycosuria may increase, but in favourable cases it soon diminishes and may disappear altogether while the patient is on the oatmeal diet, and, in some cases, the urine may remain sugar-free even when the patient returns to a restricted diet. The resumption of a general diet, or of a meat-fat-vegetable diet, must however be gradually made, and for a long time, often for months, after the exclusive oatmeal feeding has been stopped, oatmeal should still remain the only carbohydrate taken. A mixture of carbohydrate should be carefully avoided. Animal protein should also be resumed with care ; for even in favourable cases, but more especially in those where the excretion of a sugar and acetone was only reduced, the addition of animal proteins is promptly followed by increased glycosuria and an increased excretion of acetone bodies. Crofton recommends that, as a precautionary measure against acidosis, from two to four teaspoonfuls of sodium bicarbonate alone, or mixed with equal parts of magnesia usta, should be given daily throughout the oatmeal treatment. Von Noorden found that of three hundred and ten patients who had taken a systematic oatmeal cure only sixty-five were not benefited, but that in thirty-five the condition was aggravated. Blum states that he obtained excellent results with thirty-five cases, the success of the cure depending on the intensity of the disease. He considers that, while the ordinary dietetic methods are equally effectual in mild cases, even with these the oatmeal treatment is more rapid, is more easily managed, and is accepted better by the patient. With severe cases, where there is acidosis, he considers that the oatmeal cure is of great use, but insists that not more than 75 grams of oatmeal should be given for a few days at first, and then a vegetable day should be prescribed. The most brilliant results were obtained by Crofton in children, particularly when the oatmeal cure was started as soon as possible after the glycosuria was discovered. Cases of long duration did not fare so well. He considers that in adults the treatment is worse than useless when they are of a mild type and can still utilise some carbohydrate, but that when the

sugar does not disappear from the urine, even after some time on a carbohydrate-free diet, the effect is often favourable, although the results are not as satisfactory as with many children. As a rule the improvement is only temporary, and is not maintained when the patient returns to an ordinary restricted diet. Most authorities are agreed that the oatmeal cure when carefully controlled, and when employed in properly selected cases, is a useful and often indispensable adjuvant in the treatment of diabetes, but that when it is applied promiscuously it is distinctly dangerous. It has been pointed out by Minkowski that the oatmeal diet causes a tendency to water retention within the organism, and that œdema and partial retention of urine sometimes result, these symptoms disappearing, however, when it is discontinued.

No very satisfactory explanation of the beneficial effects that the oatmeal cure undoubtedly exerts in some cases of diabetes has yet been advanced. Some consider that it is not due to any specific action of the oatmeal, but depends upon the lowering nature of the diet and the restriction of protein, more particularly the absence of albuminous substances of animal origin, which not only does away with a source of sugar production, but also reduces the metabolism as a whole. Blum states that wheatmeal given in a similar way to oatmeal is just as efficacious, and Gründ found that barley-meal gives similar therapeutic results. The experiments of Rolly also suggest that there is no essential difference between these various forms of starch. Magnus-Levy, while he asserts that the advantages of oatmeal are mostly on the negative side, and are largely due to absence of meat, admits that oatmeal starch appears to have some special feature which renders it superior to the starch of other grain foods. This might be due to a difference in constitution, or to the presence in the oat grain of some substance that favours the utilisation of its starch. He found that pure oat starch, freed from other substances contained in oats, given with eggs and sanatogen, was just as efficacious as ordinary oats, so that the superiority of oatmeal in the treatment of diabetes must be ascribed to a peculiarity of the starch itself. It has always been assumed that starch, whatever its origin, is always the same substance, but if the results of these observations be confirmed, it would appear that this is not the case, and that the varying effects of other starchy foods in glycosuria may be partly due to differences in composition as yet unrecognised. Naunyn has suggested that the effects of oatmeal may be due to its not being utilised as sugar, and, with Magnus-Levy, believes that oat starch undergoes some peculiar transformation through the action of micro-organisms in the alimentary tract whereby it is converted into fermentation

products rather than into simple sugars, as is ordinarily the case with starch. Falta has expressed the opinion that the action of oatmeal is specific and is not dependent in any way upon a restriction, or difference in kind, of the protein content of the diet. A series of experiments by Hunt have shown that the resistance of mice to the poisonous effects of aceto-nitrile is very much increased by an oatmeal diet, probably owing to a specific effect exerted on the thyroid gland, and it is consequently possible that it may be by some such action that it produces its effect on metabolism in diabetes.

The recent discovery of the effects of the removal of the cuticle of rice in producing beri-beri, and the influence on the disease of giving the pericarp of the cereal, suggest that there may be some part of the potato, of oats, and of rice which has a specific action on metabolism, and which if it can be isolated from the cereals or from the preparations of diastase, may prove to have specific action in the treatment of disease. Von Noorden has suggested that the diminished glycosuria that results from the oatmeal treatment may depend upon an alteration in the permeability of the kidneys, and experiments carried out by Barrenscheen on the delay in the excretion of milk-sugar injected intravenously into non-diabetic subjects caused by an oatmeal diet tends to support this hypothesis.

Strauss and others have advocated the use of vegetables rich in inulin, such as artichokes, dandelion, the roots of black viper's grass, and edible species of the sunflower family, in diabetes. It is claimed that the absorption of inulin from such vegetables takes place very slowly, and that it is a form of carbohydrate that is well borne by many diabetics. The value of inulin as a source of energy, has, however, been questioned by Lewis, who concluded that the quantities that can be utilised are insignificant. His experiments showed that inulin taken by the mouth is partly converted by the acid of the gastric juice into levulose, and it is only this that can be made use of, for any that leaves the stomach unchanged undergoes bacterial decomposition in the intestine with the formation of gas, but no sugar, and that the inulin that escapes the bacterial change appears in the fæces. The extent to which inulin can be utilised depends therefore on the acidity of the gastric juice, and to a certain extent on the diet, and consequently varies very much in different individuals and in the same person under different conditions.

The Energy Requirement in Diabetes.—In consequence of the serious loss of strength and marked emaciation met with in severe cases of diabetes it might be thought that larger quantities

of food are necessary than in health, and the fact that there is often a voracious appetite would appear to confirm this. The experiments of most investigators have shown, however, that the energy requirement is little, if at all, altered, for the diabetic condition does not involve a decrease in the quantity of energy produced, but only an alteration in its source. The early experiments of Pettenkofer and Voit showed no change in the metabolism in diabetes from the normal. Rubner, using phlorhidzinised dogs, found that the heat production was increased by 7 per cent. when glycosuria was produced, but as the protein metabolism was raised more than threefold, he attributed this alteration in energy production to the specific dynamic action of the increased protein metabolism. Falta could find no evidence that the energy requirement is increased in diabetes, and Du Bois and Veeder showed that the amount of energy required by diabetics is approximately 34 calories per kilogram of body-weight, which is not greater than for a healthy man. Benedict and Joslin, as the result of a series of metabolic experiments on thirteen cases representing various types of glycosuria, state that the heat production may be 15 per cent. above the normal. Some observers have contended, on the other hand, that in severe diabetes there is a lowered energy requirement. Kolisch, for instance, states that 25 calories per kilogram may lead to an increase in body-weight, and that in some instances as little as 20 calories are sufficient to maintain the patient's condition. He strongly advises a minimum amount of food, and claims that this offers the best hope of success in the treatment of severe cases.

More than a hundred years ago Prout pointed out the great advantage of limiting the quantity of food in diabetes, and it is now generally agreed that high-feeding is injurious, and that in most cases the best results are obtained by arranging the diet so that the normal 34 to 35 calories per kilogram of body-weight are supplied, due allowance being made for the potential energy lost in the sugar contained in the urine. Some severe cases of diabetes, however, do best when the minimum amount of food that will maintain the body-weight is given. As a rule, not more than 100 to 120 grams of protein should be taken in the day, and this amount should be reduced on the appearance in the urine of signs of acidosis.

BIBLIOGRAPHY

Atwater, *Amer. Journ. of Physiol.*, 1904 ; *Proc. Amer. Phys. Soc.*, xxx.
Atwater and Benedict, *Memoirs of the Nat. Acad. of Sci.*, 1902.
Atwater and Bryant, *Report Storr's Agricult. Expt. Station*, 1899.
Barrenscheen, *Biochem. Zeit.*, 1912.

Benedict and Joslin, quot. Lusk, *Journ. Amer. Med. Assoc.*, 1910.

Blum, *Semaine Médicale*, 1911.

Du Bois and Veeder, *Arch. f. int. Med.*, 1910.

Chittenden, *Physiol. Economy of Nutrition*, 1894.

Cremer, *Zeit. f. Biol.*, 1901 ; *Münch. med. Woch.*, 1902.

Crofton, *Journ. Amer. Med. Assoc.*, 1909.

Falta, *Wien klin. Woch.*, 1909 ; *Germ. Cong. Int. Med.*, 1911.

Geelmuyden, *Zeit. f. phys. Chem.*, 1897.

Gründ, *Zeit. f. phys. Chem.*, 1902 ; *Germ. Cong. Int. Med.*, 1911.

Hunt, *Journ. Amer. Med. Assoc.*, 1911.

Von Jaksch, *Deut. Arch. f. klin. Med.*, 1899.

Johansson, Billstrom, and Heyl, *Skan. Arch. f. Physiol.*, 1904.

Joslin, *Journ. of Med. Res.*, 1904.

Kolisch, *Zeit. f. phys. u. diat. Therap.*, 1908.

Landergren, *Skan. Arch. f. Physiol.*, 1903.

Lee and Harrold, *Amer. Journ. of Physiol.*, 1900.

Lehmann and Voit, *Zeit. f. Biol.*, 1900.

Lewis, *Journ. Amer. Med. Assoc.*, 1912.

Lindemann and May, *Deut. Arch. f. klin. Med.*, 1896.

Lusk, *Zeit. f. Biol.*, 1890, 1901 ; *The Science of Nutrition*, 1906.

Lüthje, *Münch. med. Woch.*, 1902.

Magnus-Levy, *Arch. f. Physiol.*, 1904 ; *Arch. f. exp. Path. u. Pharm.*, 1899 ; *Berl. klin. Woch.*, 1911.

Meissel and Strohmer, *Sitzungsber. a. k. Acad. d. Wissensch.*, 1883.

Mendel and Lusk, *Amer. Journ. Physiol.*, 1903 ; *Deut. Arch. f. klin. Med.*, 1904.

Minkowski, *Arch. f. exp. Path.*, 1893 ; *Germ. Cong. Int. Med.*, 1911.

Miura, *Zeit. f. klin. Med.*, 1892.

Munk, *Arch. f. Physiol.*, 1891.

Neubauer, *Münch. med. Woch.*, 1905; *Arch. f. exp. Path. u. Pharm.*, 1909.

Neuberg, *Ergeb. d. Physiol.*, 1904.

Von Noorden, Herter Lectures on "Diabetes," 1905 ; *Handb. d. Ernahrungstherapie*, v. Leyden, 1904 ; *Diabetes*, 1905.

Pettenkofer and Voit, *Zeit. f. Biol.*, 1867.

Reilly, Nolan, and Lusk, *Amer. Journ. of Physiol.*, 1895, 1898.

Rolly, *Deut. Arch. f. klin. Med.*, 1912.

Rosenfeld, *Ergeb. d. Physiol.*, 1903.

Rosenheim, *Arch. f. Physiol.*, 1891.

Rubner, *Zeit. f. Biol.*, 1885 ; *Gesetze d. Energieverbrauchs*, 1902 ; v. Leyden's *Handbuch*, 1903.

Satta, *Hofmeister's Beitr.*, 1904–5.

Schulz, *Pflüger's Arch.*, 1896.

Schwarz, *Deut. Arch. f. klin. Med.*, 1903.

Sieven, *Skan. Arch. f. Physiol.*, 1901.

Stiles and Lusk, *Amer. Journ. Physiol.*, 1903.

Stohmann, *Journ. of prakt. Chem.*, 1885.

Voit, *Physiol. d. Stoffwechsels u. d. Ernahrung*, 1881.

Voit and Korkunoff, *Zeit. f. Biol.*, 1895.

Zuntz and Heineman, *Pflüger's Arch.*, 1900.

The Dietetic Treatment.—The aim of the modern dietetic treatment of diabetes is to so balance the diet that as perfect nutrition as possible is maintained, without there being undue strain on the organs of metabolism in any direction. The administration of carbohydrates in larger amounts than the defective metabolic powers of the diabetic organism can deal with is undoubtedly injurious, but their permanent exclusion from the diet is even more harmful, and is often quite unnecessary. A temporary exclusion is, in some instances, advisable and beneficial, for not only is the excess of sugar in the blood thereby diminished, so that the cause of many of the troubles to which diabetics are liable is lessened, or abolished, but at the same time the metabolic powers, like a released spring, often recover their tone to a surprising extent, and the tolerance for carbohydrates is materially increased. Similar results are obtained in other cases by merely restricting the carbohydrate intake, both in quantity and kind, until the glycosuria is controlled. Some diabetic patients have a marked susceptibility to proteins, which as we have seen may yield 45 to 60 per cent. of sugar, so that their glycosuria is decreased more by diminishing the protein and allowing a certain amount of carbohydrate in the diet, than by excluding the latter altogether. The vegetable proteins are, as a rule, found to be less harmful than those of meat, and consequently periods of vegetable feeding may be advantageously introduced into the treatment. For the same reason prepared vegetable proteins, &c., can often be used in place of a corresponding amount of meat in the ordinary diabetic diet. It must always be borne in mind that some diabetics never become permanently sugar-free, and that an attempt to make them so is liable to give rise to serious consequences. Even in the most severe cases there is some stored glycogen in the tissues, but if this is used acidosis will quickly develop, and the power to store further glycogen be lost or markedly diminished. It is therefore better to maintain a fairly liberal supply of glycogen in the body and a low sugar-content in the urine than to attempt to establish a sugar-free condition of the urine with the attendant risk of a

greatly depleted glycogen store in the tissues. No routine line of treatment, however sound it may be in principle, can be applied to all cases. The metabolic powers of each patient must be experimentally determined and a diet adapted to his requirements be worked out, and not only so, but it must be adjusted from time to time to meet the altering requirements of the case. That is to say, we must treat the patient, not the disease.

In working out a diet the first point to determine is the gravity of the case. For this purpose we must ascertain, first, the intensity of the diabetes ; secondly, the presence and degree of any secondary abnormalities of metabolism that may exist ; and, thirdly, whether any disturbance of nitrogenous equilibrium is present. To this end the urine passed in twenty-four hours is collected, starting and finishing the collection at an early morning hour before food has been taken. It is then carefully measured, and the total excretion of sugar, nitrogen, and ammonia nitrogen is worked out. In order that an accurate estimate may be formed of the power the patient possesses of dealing with sugar, that is to say, the intensity of the diabetes, it is necessary that the intake as well as the output should be known. The latter is given by the urinary analysis. For the former the patient must be put upon a diet of known composition, both as regards kind and quantity, such as that shown in the following table (p. 308), for forty-eight hours before the collection is made. This diet contains 102 grams of carbohydrate, but as, in severe cases of diabetes, dextrose can also be derived from protein, the possible sugar from this source must also be taken into account in calculating the total sugar value of the diet. Now the 114·2 grams of protein that it contains are equivalent to 18·27 grams of nitrogen $\left(\frac{114·2}{6·25}\right)$; and since, at the most, each gram of nitrogen can be accompanied by 5 grams, or, according to Lusk, 3·65 grams, of dextrose, the possible yield of sugar from this amount of protein would be 91·35 grams (18·27 × 5), or 66·68 grams (18·27 × 3·65), according to whichever figure is accepted. We therefore find that the total sugar value of this particular diet is approximately 169 grams, or possibly 193 grams.

(1.) The amount of carbohydrate contained in the diet as such will influence the output of dextrose in the urine within a short time after it has been taken, but the protein food does not necessarily affect it at once—moreover, dextrose may be formed from the tissues, so that the quantity of sugar of protein origin appearing in the urine will be governed by the intensity of protein metabolism, and does not necessarily bear a direct relation to the protein content of the food. Since the total nitrogen content of the urine can be

Test Diet

Grams.	Oz.		Protein.	Fat.	Carbohy.	Calories.
225	8	Coffee (1 large cup) . . .	...	...	...	...
20	¾	Cream (1 tablespoonful) . .	0·7	5·1	0·7	54
50	2	Egg (1 average) . . .	6·6	6·0	...	83
56	2	Bacon (smoked, weighed uncooked)	5·6	39·0	...	378
15	½	Margarine	0·2	11·0	...	111
45	1½	White bread (1 thick slice) . .	3·9	0·5	22·3	112
28	1	Sardines (3 average) . .	6·3	5·4	...	77
28	1	Lettuce or endive . . .	0·3	0·1	1·0	5
28	1	Tomato	0·4	0·1	1·3	7
22	¾	French dressing {4 tablesp. ol. oil / 1 „ vinegar / ¼ teasp. salt, pepper} (2 dessertsp.)	...	16·0	...	148
18	¾	Yolk of egg	2·9	6·0	...	68
96	3¼	Mutton (roast) . . .	25·2	21·7	...	300
43	1½	White bread . . .	3·9	0·5	22·3	112
15	½	Margarine	0·2	11·0	...	111
225	8	Milk (1 glass) . . .	7·3	8·8	11·0	157
56	2	White fish (cod, hake, sole, whiting)	12·0	0·2	0·12	50
28	1	Bacon	2·8	19·5	...	189
84	3	Beef (roast) . . .	18·7	24·0	...	300
150	5¼	Potato (boiled, 1 medium-sized)	3·7	0·1	31·4	144
56	2	Spinach or tomato . .	1·3	0·2	1·5	9
15	½	Margarine	0·2	11·0	...	111
14	½	White bread . . .	1·3	0·2	7·5	37
28	1	Cheddar cheese . . .	7·7	10·3	1·2	153
14	½	Brazil nuts (4 large) . .	2·3	9·3	1·0	100
20	¾	Cream (1 tablespoonful) .	0·7	5·1	0·7	54
225	8	Coffee	...	...	...	...
			114·2	211·1	102·1	2850

$$
\begin{aligned}
\text{Carbohydrate} & \quad . \quad . \quad . \quad 102\cdot1 \text{ grams} \\
\text{Protein sugar} & \left(\frac{114\cdot2}{6\cdot25} \times 5 \right) \quad . \quad 91\cdot4 \quad ,, \\
\text{Total sugar value} & \quad . \quad . \quad 193\cdot5 \text{ grams}
\end{aligned}
$$

taken as an index of protein metabolism of the body, the quantity
of nitrogen found to be present in any particular twenty-four hours'
sample multiplied by 5 (or 3·65) will give the possible sugar that
can be derived from that source, and this, plus the amount of carbo-
hydrate known to have been consumed, will represent the sugar
that might appear in the urine if the power of metabolising carbo-

hydrate were completely lost. The relationship between the sugar that might be excreted, and that which actually appears in the urine, can be expressed in a single term by dividing the latter by the former, but as this would give an inconvenient fraction, or decimal, Falta has suggested that the result should be multiplied by 100, which will give the percentage of available sugar that is eliminated unutilised. This he terms the " Coefficient of Excretion."

Falta's Coefficient of Excretion =

$$\frac{\text{Total dextrose in urine} \times 100}{(\text{Total urinary nitrogen} \times 5) + \text{food dextrose}}$$

If Lusk's factor is used the formula will be :—

Lusk's Coefficient of Excretion =

$$\frac{\text{Total dextrose in urine} \times 100}{(\text{Total urinary nitrogen} \times 3 \cdot 65) + \text{food dextrose}}$$

Slightly higher figures will of course be obtained when Lusk's formula is used than when Falta's is employed ; but this is of little practical importance, as the numbers are not absolute values, and are only useful as an index of the severity of the diabetes by which one case can be compared with another and the progress of the same case can be accurately gauged from time to time, in much the same way as the severity and progress of an anæmia can be estimated and watched by blood-counts and hæmoglobin estimations, or the course of a fever can be followed with the thermometer.

(2.) Of the secondary disturbances of metabolism that occur in diabetes the most important is the acidosis that indicates imperfect oxidation of fats and proteins and that is the forerunner of diabetic coma. The presence of aceto-acetic acid in the urine shows that this stage in the progress of the case has been reached, but for accurate work it is necessary that an approximate idea at least should be obtained of the extent of the defect. A rough measure of the degree of acidosis is given by the amount of sodium bicarbonate that must be administered before the ferric chloride reaction in the urine disappears, or the quantity in excess of 2 drams required to make the urine alkaline. But the simplest way is to estimate the ammonia nitrogen in the urine. This is not strictly accurate, but for routine clinical work it gives as near an approximation as is necessary.

(3.) According to Weintraud, acidosis is only dangerous when it is accompanied by a disturbance of nitrogenous equilibrium, that is to say, when more nitrogen is excreted than is absorbed, so that a determination of the total urinary nitrogen is important,

not only for the estimation of the possible sugar output, but also
as a guide to the significance to be attached to the presence of the
acetone bodies and an excess of ammonia nitrogen in the urine.
The state of nitrogenous equilibrium is ascertained by balancing
the nitrogen-content of the food, estimated from diet tables, and
allowing 1 gram of nitrogen for each 6·25 grams of protein, as shown
above, against the nitrogen excreted in the urine, plus about 1 gram
to represent the unabsorbed nitrogen contained in the fæces.   More
accurate results could of course be obtained by making complete
analyses of the food and excreta, but since a difference of 1 or 2
grams only is of no practical importance the method suggested is
found to be sufficiently reliable.

Having by these preliminary investigations obtained informa-
tion as to the type of case with which we have to deal, the next step
is to work out a diet suitable to the conditions found.   For this
purpose it is necessary to ascertain (1) how much, and what kinds of
carbohydrate can be used with safety to supply as much as possible
of the energy required, (2) what amount of protein is needed to
maintain nitrogenous equilibrium, and (3) how much fat must be
added to the diet to make up the balance of the energy requirement.
The answers to these questions can only be obtained by metabolic
experiments controlled by analyses of the urine and careful regular
weighing of the patient.

If the patient has a low coefficient of excretion, the output of
ammonia nitrogen is not high, and nitrogenous equilibrium is not
seriously disturbed, he is at once put on a " carbohydrate-free " diet
—that is to say, upon a diet that consists almost entirely of proteins
and fats, such as that given on the opposite page.

Such a diet is not strictly carbohydrate free, for it contains
9 grams, chiefly in the form of vegetables.   If it is thought advisable
these may be omitted ; but they form a useful vehicle for a consider-
able amount of fat, and, as part of the raw starch is probably not
absorbed, the diet is as free from carbohydrates as it can con-
veniently be made and yet remains palatable.

After the patient has been forty-eight hours on this diet the
urine is collected and tested for sugar, acetone, aceto-acetic acid, &c.,
and the total amount of sugar, ammonia nitrogen, and total nitrogen
determined.   If the glycosuria has disappeared, or after a few more
days on the diet it does so, as it probably will, gradually increased
quantities of white bread—starting, say, with half an ounce (14 grams)
—are given each day until traces of sugar reappear.   The amount of
bread which produces this effect indicates the patient's limit of
tolerance for this particular form of starch.   He is then again
placed on a carbohydrate-free diet for twenty-four hours, and his

"*Carbohydrate-free*" *Diet*

Grams.	Oz.		Protein.	Fat.	Carbohy.	Calories.
225	8	Coffee (1 large cup) . . .	...	...	...	...
18	¾	Cream (1 tablespoonful) . .	0·7	5·1	0 7	54
50	2	Egg (1 average)	6·6	6·0	...	83
56	2	Bacon (smoked, weighed uncooked)	5·6	39·0	...	378
14	½	Margarine	0·2	11·0	...	103
28	1	Kalari biscuits (Callard), 7 biscuits	17·0	7·5	...	107
18	¾	Yolk of one egg	2·9	6·0	...	68
28	1	Lettuce or endive . . .	0·3	0·1	1·0	5
28	1	Tomato	0·4	0·1	1·3	7
22	¾	Dressing (2 dessertsp.) { ¼ tablesp. ol. oil / 1 ,, vinegar / Salt, pepper	...	16·0	...	148
56	2	White fish (cod whiting, hake, &c.)	12·0	0·2	0·12	50
28	1	Bacon (smoked, weighed uncooked)	2·8	19·5	...	189
28	1	Cheese (Cheddar	8·0	11·0	1·3	142
14	½	Margarine	0·2	11·0	...	103
28	1	Kalari biscuits (Callard), 7 biscuits	17·0	7·5	...	107
18	¾	Cream (1 tablespoonful) . .	0·7	5·1	0·7	54
225	8	Coffee (1 large cup) . . .	...	...	...	...
28	1	Sardines (3 average) . . .	6·3	5·4	...	77
28	1	Lettuce or endive	0·3	0·1	1·0	5
28	1	Tomato	0·4	0·1	1·3	7
18	¾	Yolk of one egg	2·9	6·0	...	68
22	¾	Dressing as above (2 dessertspoon-fuls)	...	16·0	...	148
98	3½	Roast-beef or mutton . . .	22·3	28·6	...	357
18	¾	Cognac	...	(8·4)	0·1	70
14	½	Brazil nuts (4 large) . . .	2·3	9·3	1·0	100
18	¾	Cream (1 tablespoonful) . .	0·7	5·1	0·7	64
225	8	Coffee (1 large cup) . . .	...	...	...	...
			109·6	**224·1**	**9·3**	**2494**

$$\begin{aligned} \text{Carbohydrate} \quad . \quad . \quad . \quad &. \quad 9·3 \text{ grams} \\ \text{Protein sugar}\left(\frac{109·6}{6·25} \times 5\right) \quad &. \quad 87·5 \quad ,, \\ \text{Total sugar value} \quad . \quad &. \quad \underline{96·8} \text{ grams} \end{aligned}$$

limit of tolerance for other sugars and starches determined by adding equivalent quantities of dextrose, levulose, oatmeal, potato, milk, &c., a day on a carbohydrate-free diet being interposed between each test. The quantities of the commoner starchy and sugar foods containing the same amount of carbohydrate as one ounce of bread are shown in the following table :—

Grams	Oz.	Carbohydrate Equivalents of 1 oz. (30 grams) Bread.				Protein. Grams.	Fats. Grams.	Carbohy. Grams.	Calories.
30	1·0	Bread, white	.	.	.	2·8	0·4	15·9	80
26	0·8	,, toasted	.	.	.	3·0	0·4	15·9	90
34	1·0	,, brown	.	.	.	1·8	0·6	15·9	78
32	1·0	,, wholemeal	.	.	.	3·1	0·3	15·9	81
32	1·0	,, gluten	.	.	.	3·0	0·5	15·9	82
72	2·4	Farina	.	.	.	1·2	0·1	15·9	41
21	0·7	Force	.	.	.	1·9	0·3	15·9	75
20	0·7	Grape-nuts	.	.	.	2·4	0·1	15·9	77
90	3·0	Hominy (boiled)	.	.	.	2·0	0·2	15·9	75
100	3·3	Macaroni(,,)	.	.	.	3·0	1·5	15·9	91
24	0·8	Oatmeal (dry)	.	.	.	3 8	1·7	15·9	97
138	4·6	,, (boiled)	.	.	.	3·9	0·7	15·9	87
65	2·2	Potato (baked)	.	.	.	1·9	0·1	15·9	74
75	2·5	,, (boiled)	.	.	.	1·8	0·07	15·9	73
34	1·1	,, (chips)	.	.	.	2·3	13·6	15·9	202
65	2·2	Rice (boiled)	.	.	.	1·8	0·07	15·9	73
21	0·7	Shredded wheat biscuits	.	.	.	2·2	0·3	15·9	77
170	5·7	Vermicelli	.	.	.	2·4	0·4	15·9	80
16	0·5	Dextrose	.	.	.	...	...	16·0	66
16	0·5	Levulose	.	.	.	...	...	16·0	66
317	10·5	Milk, whole	.	.	.	10·7	12·7	15·8	226
319	10·6	,, whey	.	.	.	3·2	1·0	15·9	88

By comparing the coefficients of excretion, and the total amounts of ammonia nitrogen, one with the other, the effects of each of these substances on sugar excretion and on metabolism can be determined. The results are more readily appreciated if they are plotted out on squared " graph " paper, using different coloured inks, or forms of line, to represent the intake and output of carbohydrate, the total available carbohydrate in the diet, the coefficient of excretion, the ammonia nitrogen, the total nitrogen, quantity of urine, &c. &c. (Chart I.).

In this chart only the carbohydrate content of the food (F.S.), the sugar excreted in the urine (U.S.), the coefficient of excretion (C.E.), and the ammonia nitrogen (multiplied by ten) (Am. N.) are shown, to avoid confusion. It will be seen that the patient could take 22 grams of carbohydrate, including half an ounce of white bread, without producing glycosuria, but that when the carbohydrate content of the diet was raised to 30 grams by adding another half ounce of bread, 5·5 per cent. of the available sugar appeared in the urine unused, while 68 grams of carbohydrate, including 3½ ounces of bread, gave a coefficient of excretion of 14 per cent. Of the other carbohydrates tried in this case only potato, milk, and levulose appeared to be assimilated with anything like ease. It will be noticed that when the patient was placed on a " carbo-

hydrate-free " (test) diet there was a slight rise in the excretion of ammonia nitrogen, but on adding carbohydrate this fell to below the former level. At no time, however, did it exceed the normal

F.S. = Food Carbohydrate.   U.S.= Sugar in Urine.

C.E. = Coefficient of Excretion.   Am. N.= Ammonia Nitrogen × 10.
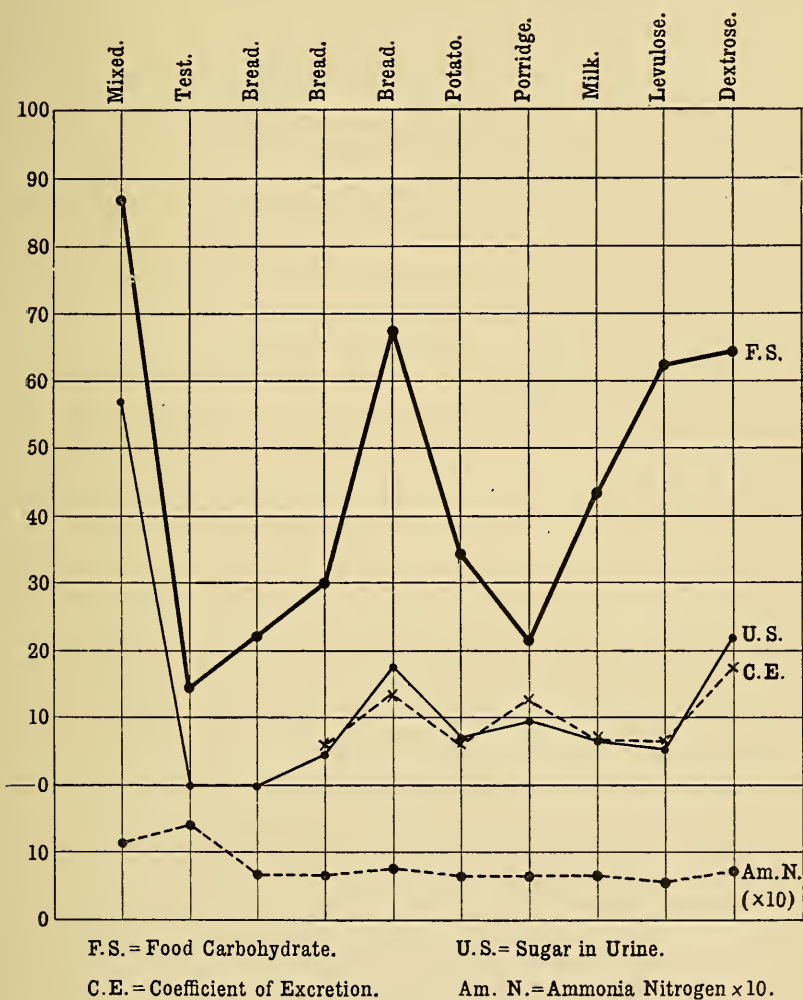
CHART I.

limit, and the patient's urine never contained any aceto-acetic acid, though traces of acetone were present throughout, excepting when potato was being taken.

It will generally be found that, as in the above case, some forms

of carbohydrate are better borne than others, and that not only is a smaller proportion excreted in the urine as sugar, but that the tendency to acidosis is reduced. By selection one or more of those which appear to be best suited to the idiosyncrasies of the case, and by giving them in quantities which experiment has shown to be below the toleration point, considerable variation in the diet can often be arranged, a most important consideration from the patient's point of view, and one which generally ensures his more strict adherence to the directions given him. In the case just mentioned, for instance, the patient was allowed half an ounce of white bread, or half a pint of milk, or half an ounce of levulose, with occasionally an ounce of potato. Only one form of carbohydrate should be allowed on one day, and those which are best borne should be most frequently taken. It is essential that the patient should be impressed with the absolute necessity of his not exceeding the quantity of each that is prescribed for him. The time of day at which starchy foods are taken appears to exert some influence upon their effects upon metabolism, as a rule they are best tolerated with an early morning meal.

Having determined the caloric value of the types and quantity of carbohydrate allowable, the diet is completed by adding sufficient protein to produce from 1·5 to 1·6 calories per kilogram of the body-weight, and then making up the balance of energy to 34 or 35 calories per kilogram with fatty foods. Here, again, the diet may be varied by classifying the commoner foodstuffs in groups, according to whether they are preponderatingly protein or fatty, and subdividing each group so that foods of a similar type are collected together. If we then work out the quantity of each which will yield a definite number of calories, this unit for convenience of reference being termed a " ration," we can substitute one for the other as convenience or appetite may direct, and yet be sure that the patient is receiving the required amount of energy. The " ration " I have selected for the unit is an average serving of roast-beef, which weighs 3 ounces, and yields 300 calories. Any of the other rations shown in the tables (pp. 315, 316) may be substituted for this, for they all yield approximately the same amount of energy.

It will be noticed that some of the rations are inconveniently large, and also that in the first table some contain an amount of protein much in excess of that in a ration of roast-beef. To get over this difficulty the quantities actually given are those shown in the column headed " portion allowed," in which a fraction of the whole ration is indicated. By combining several such fractional portions the food value of a whole ration can be obtained without giving an excess of any one food or too large an amount of protein.

TABLE I

Grams.	Oz.	Protein "rations" yielding approximately 300 Calories.	Protein.	Fat.	Carbohydrates.	Calories.	"Portion Allowed."
84	3	1 slice beef (roast)	18·7	24·0	...	300	1
105	3¾	3 slices ham (smoked, boiled) . .	23·4	21·2	...	300	½
150	5¼	2 ,, lamb (roast)	29·5	19·0	...	300	½
96	3⅜	1 ,, mutton (roast) . . .	25·2	21·7	...	300	1
143	5	1½ ,, pork (roast)	40·6	14·3	...	300	½
100	3½	4 ,, tongue (tinned) . . .	19·5	23·2	...	300	⅓
232	8¼	3 ,, veal (roast)	65·9	3·0	...	300	⅓
166	5¾	1½ average help. chicken, grouse, partridge, pheasant, pigeon (roast) .	53·3	2·9	...	300	½
130	4⅝	2 ,, duck, goose (roast) .	17·7	25·2	...	300	½
105	3¾	1 ,, turkey (roast) . .	29·2	19·3	...	300	½
306	3¾	3 average help. cod, haddock, hake, plaice, sole, whiting . .	66·3	1·1	6·00	300	¼
248	8¾	2½ ,, halibut . . .	50·5	10·0	...	300	½
202	7	3 ,, mackerel . . .	35·2	12·5	7·56	300	½
152	5¼	1½ ,, salmon . . .	30·1	15·5	8·15	300	½
107	3¾	10 average fish, sardines (tinned) .	24·5	11·0	...	300	½
350	8¼	25 ,, smelts . . .	55·9	7·2	1·5	300	⅙
263	9¼	5 ,, trout (brook) . .	55·6	8·1	3·26	300	⅙
200	7	4 average eggs, whole . . .	26·8	21·0	...	304	1
90	3¼	5 ,, ,, yolks . . .	14·5	30·0	...	330	1

TABLE II

Fat "Rations" yielding approximately 300 Calories (in Order of Protein Contents).

Grams.	Oz.		Protein.	Fat.	Carbohydrate.	Calories.	"Portion Allowed."
33	1·1	lard	...	33·0	...	300	1
33	1·1	suet	...	33·0	...	300	1
33	1·1	2½ teasp. olive-oil	...	33·0	...	300	1
40	1·4	3 pats butter	0·4	32·3	...	300	1
40	1·4	,, margarine	0·5	32·3	...	300	1
48	1·6	Devonshire cream	2·0	31·2	0·4	300	½
111	3·7	5½ tablesp. cream	4·1	28·5	4·0	300	½
46	1·5	2 slices bacon (weighed uncooked)	4·8	29·8	...	300	1
42	1·4	7 large Brazil nuts	7·1	28·0	3·0	300	½
45	1·5	30 ,, almonds	9·4	24·7	7·8	300	⅓
135	4·5	15 ,, green olives	10·5	27·3	11·7	300	⅛
65	2·2	6 scoopfuls cheese, Stilton	15·4	25·3	...	300	½
63	2·1	3 teasp. ,, Cheddar	17·4	23·2	2·6	300	¼
80	2·7	4 cub. in. ,, Roquefort	18·0	23·6	1·4	300	½
70	2·3	3 tablesp. cream cheese	18·3	23·6	1·7	300	¼
75	2·5	4 teasp. cheese, Cheshire	20·2	23·0	0·7	300	½

When a patient has had a diet worked out for him he is told how much energy he requires a day, and is then given a specimen diet with a list of various foods that he may substitute for those shown. To avoid the inconvenience of manipulating fractions I am in the habit of taking the "food value" of a unit ration as 100 and allotting to the other foods corresponding values, so that the diet chart for proteins and fatty foods given to the patient reads as follows :—

Meat— Food Value.

    Beef 3 oz., mutton $3\frac{1}{4}$ oz. 100

    Lamb $2\frac{1}{2}$ oz., pork $2\frac{1}{2}$ oz., ham $1\frac{3}{4}$ oz., tongue $1\frac{3}{4}$ oz. . . 50

Poultry—

    Chicken 3 oz., grouse 3 oz., partridge 3 oz., pheasant 3 oz.,

      pigeon 3 oz., duck $2\frac{1}{4}$ oz., goose $2\frac{1}{4}$ oz., turkey 2 oz. . . 50

Fish—

    Halibut 4 oz., mackerel $3\frac{1}{2}$ oz., salmon $2\frac{1}{2}$ oz., sardines

      (tinned in oil) 2 oz. 50

    Cod $2\frac{1}{2}$ oz., haddock $2\frac{1}{2}$ oz., hake $2\frac{1}{2}$ oz., plaice $2\frac{1}{2}$ oz., sole

      $2\frac{1}{2}$ oz., whiting $2\frac{1}{2}$ oz. 25

    Smelts $2\frac{1}{2}$ oz., trout 2 oz. 20

Fatty Foods—

    Bacon $1\frac{1}{2}$ oz., butter $1\frac{1}{2}$ oz., margarine $1\frac{1}{2}$ oz., lard 1 oz., suet

      1 oz., salad oil 1 oz. 100

    Cream 2 oz., Devonshire cream $1\frac{1}{2}$ oz., cheese (cream,

      Cheddar, Stilton) 1 oz., cheese (Cheshire, Roquefort) $1\frac{1}{2}$ oz. 50

    Brazil nuts 1 oz., olives 2 oz. 70

Eggs—

    Average whole, 4, or average yolks, 5 100

Milk—

    (Whole cream) $\frac{1}{2}$ pint 75

As a rule it will be found that three or four protein " rations " will contain about 100 to 120 grams of protein, so that this amount, yielding 900 to 1200 calories, should not be exceeded. The balance of the 1800 to 2000 calories required by an average individual must be made up in other ways. If carbohydrate tolerance is low and only a small quantity of starchy food can be given the administration of the large amount of fatty material required may present a serious difficulty, especially with some patients who have a natural repugnance to fats. Bacon, cream, and cheese will supply a considerable amount of the energy required, and are usually well taken. A few Brazil nuts, or green olives, and a certain amount of butter, or margarine, may also be given. Frequently, however, a larger quantity of the latter than most patients will tolerate when they are having little or no bread, and a certain amount of salad (olive) oil, to which many people have a strong objection,

have to be introduced into the diet. This difficulty may be over-come to a large extent by giving either oiled butter, or salad dressing, with vegetables, especially the coarser kinds. The following are the food values of several such dressings (Locke) :—

French Dressing—(4 tabsp. olive-oil, 1 tabsp. vinegar, $\frac{1}{4}$ tsp. salt, pepper)—one dessertspoonful yields 74 calories.

Hollandaise Sauce—($\frac{1}{2}$ cup butter, yolk 2 eggs, 3 teasp. lemon-juice, salt, cayenne pepper)—2 tablespoonfuls yield 170 calories.

Mayonnaise Dressing—(2 eggs, 2 cups olive-oil, 3 tabsp. vinegar, or 3 tabsp. lemon-juice, salt, pepper, mustard)—one tablespoonful yields 187 calories.

Some vegetables contain only a small proportion of carbo-hydrate, and by selecting those in which the lowest percentage (*e.g.* under 5 per cent.) is met with, and working out weights of these which contain 1 gram or less of carbohydrate, considerable choice, suitable to the tastes of the patient and the season of the year, can be offered. The commoner vegetables of this class are shown in the following table :—

The Common Vegetables containing under 5 Per Cent. of Carbohydrate

Grams.	Oz.		Protein.	Fat.	Carbohy.	Calories.
100	$3\frac{1}{2}$	1 av. helping asparagus (cooked)	1·5	0·11	2·8	18
100	$3\frac{1}{2}$	3 hpd. tablesp. French beans ,,	0·8	1·1	1·9	22
100	$3\frac{1}{2}$	3 ,, cabbage ,,	0·6	0·1	0·4	5
100	$3\frac{1}{2}$	2 ,, cauliflower ,,	0·9	0·1	0·4	7
100	$3\frac{1}{2}$	2 large mushrooms ,,	3·4	0·2	3·0	29
100	$3\frac{1}{2}$	1 average onion ,,	1·2	1·8	4·9	42
100	$3\frac{1}{2}$	4 slices parsnip ,,	0·22	0·29	1·46	10
100	$3\frac{1}{2}$	2 hpd. tablesp. spinach ,,	2·7	0·3	3·0	18
100	$3\frac{1}{2}$	2 tablesp. turnip ,,	0·3	0·06	0·6	4
100	$3\frac{1}{2}$	6 small sticks celery (raw)	1·0	1·0	2·8	16
100	$3\frac{1}{2}$	16 thin slices cucumber ,,	0·8	0·2	3·1	18
100	$3\frac{1}{2}$	endive ,,	1·0	0·2	3·0	16
100	$3\frac{1}{2}$	lettuce ,,	0·9	0·3	2·9	17
100	$3\frac{1}{2}$	radishes ,,	1·2	0·1	5·0	25
100	$3\frac{1}{2}$	sorrel ,,	2·0	2·0	3·0	25
100	$3\frac{1}{2}$	tomato .,	1·2	0·2	4·0	23
100	$3\frac{1}{2}$	watercress .,	0·7	0·0	3·7	18

The weights calculated to contain 1 gram, or less, of carbohydrate, are as follows :—

Cooked vegetables containing 1 gram or less of carbohydrate—

Food Value.

Cabbage 8 oz., cauliflower 8 oz., turnip 5 oz., parsnip 2 oz., French beans $1\frac{1}{2}$ oz., asparagus 1 oz., onions 1 oz., mushroom 1 oz. 5

Raw vegetables containing 1 *gram or less of carbohydrate—* Food Value.
    Celery 1 oz., lettuce 1 oz., endive 1 oz., sorrel 1 oz., cucumber
        1 oz., watercress 1 oz., tomato 1 oz., radishes 1 oz. .   .   2

By instructing the patient not to take more than one, or two, rations daily of these vegetables we can ensure his not deriving more than one, or two, grams of carbohydrate from this source. Although their energy value is practically negligible, they form a vehicle by which fats may be administered. They also furnish alkalies to the organism, and by their bulk they make the diet more satisfying.

In cases where there is fair tolerance for carbohydrate the diet may be further varied by allowing certain fruits which contain comparatively small percentages of carbohydrate. The more common, containing 10 per cent., or under, are as follows :—

The Commoner Fruits containing 10 *Per Cent., or under, of Carbohydrate*

Grams.	Oz.					Protein.	Fat.	Carbohy.	Calories.
100	3½	3 hpd. tablesp.	blackberries	.	.	1·30	1·00	10·00	59
100	3½	,, ,,	bilberries	.	.	1·60	0·20	7·20	32
100	3½	,, ,,	cranberries	.	.	1·30	0·10	9·90	46
130	4½	1 average	lemon .	.	.	0·90	0·85	7·67	32
100	3½	6½ teasp.	,, juice	.	.	...	...	9·80	40
128	4½	1 average	peach .	.	.	0·64	0·13	9·86	44
100	3½	Edible pt. 2 slices	pineapple	.	.	0·40	0·30	9·70	44
100	3½	2 hpd. tablesp.	rhubarb .	.	.	0·40	0·60	3·50	34
100	3½	4 hpd. ,,	strawberries	.	.	1·00	0·60	7·40	40
300	10½	1 large slice	water-melon .	.	.	0·60	0·30	8·10	39

Weights of these containing approximately 5 grams of carbohydrate are :—

Fruits containing about 5 *grams of carbohydrate—*

Food Value.

    Water-melon 6 oz., rhubarb 4½ oz., lemon 2¾ oz., bilberries
        2¼ oz., peach 2 oz., strawberries 2 oz., blackberries 1½ oz.,
        cranberries 1½ oz., lemon-juice 1½ oz., pineapple 1½ oz. .   7

The patient is also supplied with a list of foods that he must not take unless specially ordered, including :—

    Sugar and starchy food in all forms.

    Bread, toast, biscuits, pastry, pies, puddings, rice, sago, tapioca, macaroni, vermicelli, arrowroot, cornflour, oatmeal.

    Potato, carrot, turnips, parsnip, artichokes, beetroot, peas, beans, lentils.

    Fruit, sweets, chocolate, ices, jam, honey.

    Sauces and gravies thickened with flour. Thickened soups and broth.

Oysters, liver.

Milk, ale, stout, porter, cider, sweet and sparkling wines, port wine, liqueurs.

Diabetics are usually allowed meat extracts and unthickened soups, but as Thompson and Wallace have shown that the addition of even small quantities of creatinin to the diet temporarily increases the output of sugar by nearly 50 per cent., it would seem that these are best avoided.

In addition to the substances already mentioned the patient is allowed :—

Tea or coffee (without milk or sugar), vinegar, pickles, and saccharin or saxin.

A word may be said here about the use of saccharin and similar coal-tar sugar substitutes. As a rule diabetic patients are allowed an unlimited quantity, but the experiments of the Referee Board of the United States Department of Agriculture have shown that saccharin in large doses, over 0·3 grams per day, and especially over 1 gram a day, added to the food and taken for considerable periods, are liable to induce digestive disturbances, increase the free hydrochloric acid in the gastric juice, alter the reaction of the fæces, cause a greater formation of putrefactive products in the intestine, and eventually bring about serious distaste for the substance.

With regard to " diabetic " breads, and bread substitutes, it is most important that these should be obtained from a thoroughly reliable maker, and an emphatic warning must be entered against the majority of those now in the market. Many are exploited as being " practically starch-free," or as containing starch " only in a form that is readily digested and assimilated by diabetics," but analyses show that the latter have usually been merely subjected to heat, and that many of the former contain as much, or nearly as much, starch as ordinary white bread. In one analysis, for instance, the following results were obtained :—

White bread 100 grams = 9·2 grams protein, 1·3 grams fat, 53·1 grams carbohydrate.

Gluten bread 100 grams = 9·3 grams protein, 1·4 grams fat, 49·8 grams carbohydrate.

All such bread substitutes are much more expensive, both from a financial and physiological point of view, than ordinary bread, and they are really a serious danger, for by using them the patient may be unconsciously taking an amount of starch far beyond his powers of assimilation. It is better that he should be given a definite amount of a food the danger of which is known, than he should be allowed to live in a fool's paradise by substituting an

expensive proprietary preparation of unknown, and often varying, composition. In my experience very few commercial bread substitutes can be relied upon as being starch free, and those that can are usually so unpalatable that most patients soon tire and prefer to do without them. A small quantity of some thoroughly reliable diabetic bread, or biscuit, however, is often useful as a vehicle for the administration of butter, cheese, &c., but its exact composition should be known, and it should be tested for starch from time to time, otherwise it is safer to allow a definite quantity of ordinary bread.

Some people have an idea that toast is better for a diabetic than fresh bread, but a glance at the table on p. 312 will show that their composition is practically the same, the only difference being the higher proportions of protein, fat, and carbohydrate, due to the loss of water, so that 26 grams of toast are equivalent to 30 grams of fresh bread, so far as the carbohydrate content goes. The superficial layers of starch are partly converted into dextrin in the process of toasting, but this does not alter in its effect on carbohydrate metabolism.

To merely give a patient a list of foods that he " may take," and another of those that he " must avoid," is a very perfunctory way of treating a case of persistent glycosuria, and in all but the mildest forms is likely to eventually lead to disaster, or at least shorten the possible span of life. Persistent glycosuria is a disease of the chemistry of the body in much the same way as a defect of vision is an anatomical one, and just as lenses of the proper form and size are worked out for the latter, so must the diet of the former be adapted to the needs of the case, both in quality and amount. The patient must be taught that instinct is no longer a safe guide, that he must watch every mouthful that he takes, and learn that a certain amount of the foods that he can assimilate must be consumed each day. It may be thought that this is a counsel of perfection, and that most patients will not go to the trouble of weighing their food, but after a very short time it is quite unnecessary to do so, except as an occasional check, for it is surprising how quickly the eye can be trained to estimate the weights of the various foodstuffs that are required. One of the advantages of the method of working out a diet that I have outlined is that it can only be satisfactorily carried out in an institution, or nursing-home, where the food can be carefully prepared and weighed, and as the patient can at the same time be taught the quantities that he is receiving all inconvenience in this respect is avoided when he returns home. Even in mild cases of persistent glycosuria, and in many cases of transitory glycosuria, a quantitative restriction of the diet is advisable. In the more severe cases it is essential.

As the object of the treatment is to so arrange the intake of food that the metabolic powers of the patient for carbohydrates are not overtaxed, but are working below their maximum capacity, so that they may have an opportunity of recovering their tone, constant supervision is required for some time. The urine should be examined at intervals, the diet being revised accordingly to the metabolic findings, and adjusted to the progress of the case.

If faulty carbohydrate metabolism were the only factor to be considered in diabetes its treatment would be comparatively simple, but in severe cases, where there is not only a high coefficient of excretion, but also well-marked secondary disturbances of metabolism, with a marked excess of acetone bodies, a high total output of ammonia nitrogen, and a serious disturbance of nitrogenous equilibrium, we have to take into account the patient's defective powers of dealing with proteins, and probably also with fatty acids. In treating such a case we must first endeavour to restore the nitrogen balance as much as possible. This is accomplished by (1) rest in bed, (2) reducing the diuresis, (3) limiting the intake of nitrogenous food, (4) the use of such drugs as codeia, opium, arsenic, &c.

Since the large amount of urine passed is one of the characteristic features of most severe cases of diabetes, and nitrogenous equilibrium is practically impossible when more than 1200 to 1500 c.c. are excreted in a day, we must, as a preliminary step, endeavour to diminish the diuresis by reducing the glycosuria upon which it depends. The carbohydrate in the test diet is therefore cautiously reduced until the sugar disappears or is diminished, so that the daily excretion of urine falls to about 1500 c.c. As the glycosuria itself is of secondary importance, time should not be spent in the frequently futile, and dangerous, task of attempting to make the urine sugar-free, but attention should be mainly devoted, at least at first, to diminishing the diuresis sufficiently to allow of a more normal nitrogen balance being established, and to controlling the acidosis. Restriction of the carbohydrates of the food always increases the acetonuria, but if the restriction can be safely persisted in an ultimate decrease often results. A constant watch must be kept on the excretion of acetone bodies and ammonia nitrogen for indications of serious acidosis, and should there be evidence of this, coupled with the prodromal symptoms of diabetic coma, more starchy food should be added to the diet, and other means be taken to combat the condition. It is usually safe to diminish the carbohydrates in the diet, in spite of moderate acidosis, so long as there is a slight nitrogen addition to the body.

If the measures already taken are not sufficient to restore

nitrogenous equilibrium, the coefficient of excretion remains high, and the output of acetone bodies and ammonia nitrogen is ex- cessive, the next step is to reduce the amount of nitrogenous food until 100, 75, or even 50 grams of protein are being taken in the day, and not more than 12 or 15 grams of nitrogen are being ex- creted in the urine. Vegetable proteins and eggs are often found to disturb metabolism less than nitrogenous foods of animal origin, and they may be advantageously substituted for an equivalent amount of meat. Such restrictions of the protein of the food frequently exert a very favourable influence on the acidosis, and at the same time reduce the amount of sugar in the urine to a much greater extent than mere limitation of the carbohydrate, even when some starchy food is being taken at the same time. To show the manner in which the diet is worked out in severe diabetes the following example may be quoted.

The case was a serious one, with a coefficient of excretion of 84 per cent., and an ammonia nitrogen excretion of 1·2 grams in the twenty-four hours, on a diet containing 76 grams of carbohydrate, including 3 oz. of bread. On reducing the bread to 2 oz. the co- efficient of excretion fell to 66 per cent., but even with no added bread and a diet containing only 20 grams of carbohydrate, it stood at 65 per cent., and the ammonia nitrogen rose to 1·8 grams in the twenty-four hours. By cutting down the proteins of the diet the coefficient of excretion was reduced to 62 per cent., and the ammonia nitrogen excretion dropped to 1·5 grams. The tolerance for different forms of carbohydrate was tested by adding dextrose, levulose, milk, oatmeal, potato, and apple successively to the test diet, with the results shown in the chart (Chart II.).

From this it is apparent that both pure dextrose and pure levulose, particularly the latter, were utilised with difficulty, but that the carbohydrate contained in oatmeal and potato were metabolised comparatively well. The addition of milk, and potato, was found to be followed by a reduction in the output of ammonia nitrogen, so that they apparently diminished the acidosis. A diet based on these results was worked out and taught to the patient. At the end of his stay in the nursing-home he was put upon a diet contain- ing 24 grains of carbohydrate, and it was found that his coefficient of excretion had fallen to 55 per cent., as compared with 65 per cent. when the treatment was commenced, so that his power of dealing with carbohydrate had improved about 10 per cent.

In cases of this description von Noorden's " oatmeal cure " may give very satisfactory results, at least temporarily. This treatment, as we have seen, consists in the administration of 200 to 250 grams of oatmeal, 200 to 300 of butter, and 3 or 4 eggs, with

nothing else except black coffee or tea in the twenty-four hours. Such a diet will contain only from 50 to 70 grams of egg and vegetable protein, 180 to 300 grams of fat, and 135 to 170 grams of carbohydrate, yielding 1900 to 3700 calories, but as an essential part of

F.S. = Food Carbohydrate.   U.S. = Sugar in Urine.

C.E. = Coefficient of Excretion.   Am. N. = Ammonia Nitrogen × 10.
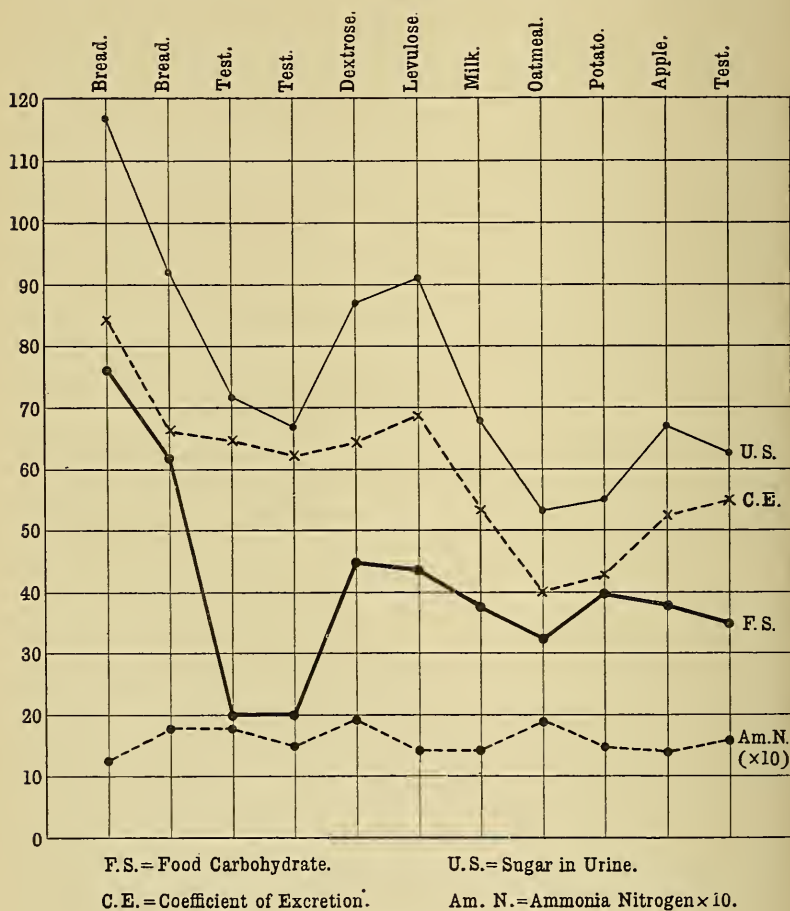
CHART II.

the treatment is the interposition of one or two days of pure vegetable diet, the nitrogenous intake is reduced to a very low level. The effect of substituting other starchy foods, such as whole wheat meal, barley meal, potato, &c., for oatmeal, may be tried, as it is sometimes found that they give as good a result, and are better liked by the patient. One form only of carbohydrate should,

however, be given at one time. Drugs, such as opium, are useful adjuncts in the treatment, when careful dieting fails to reduce the condition.

In severe cases of diabetes the quantitative arrangement of the diet is even more important than in the milder forms, for not only have we to watch the carbohydrate intake, but the amount of protein consumed must also be carefully regulated. It is, as a rule, practically impossible to keep the patient's urine free from sugar, and it is usually found that he does better when a certain amount of carbohydrate is allowed than when he is put on a strict carbohydrate-free diet. The kinds of starchy food that are best borne vary with different cases, and should be experimentally determined. Having ascertained the amounts and varieties of carbohydrate, and protein, that give the best result, we must add sufficient fat to the diet to make up the 34 or 35 calories per kilogram of body-weight required. Little or no energy for the needs of the patient can be obtained from the carbohydrate, and the proteins will probably yield but 10 or 15 per cent. of the total requirement, so that we are faced with the very serious difficulty of supplying fat in an assimilable form to make up the deficiency. This is a problem which will often tax the resources of the physician to the utmost, and requires all the arts of cookery to accomplish. Although it is now known that the acetone bodies are chiefly formed from fats, it would appear that their production in the body is not influenced by the quantity of fat in the diet unless an enormous amount is taken, so that fatty foods can be safely given in diabetes. As the lower members of the fatty acid series are more likely to cause acidosis than the higher, fatty foods that contain the latter should be selected. For this reason good margarine is preferable to butter, unless the latter has been washed to remove the butyric acid, &c., that it contains. The coarser vegetables that contain only a small proportion of carbohydrate may be used as vehicles by which a considerable quantity of salad-oil and margarine, or butter, can be introduced into the diet. Cream can be given with coffee. Bacon, cream cheese, and sardines in oil are well taken by most patients. Brazil nuts, almonds, and a few green olives may also be used in moderation, while lard and suet can be employed in various ways in the preparation of the other food materials. English patients have usually a constitutional antipathy to fatty foods, and although one may succeed for a time in prevailing on them to take a sufficient amount, they quickly tire, or gastro-intestinal disturbances super-vene which cause nausea and distaste. The latter may be pre-vented to a certain extent by giving a small quantity of alcohol with each fatty meal, but the total amount should not exceed an

alcohol content of 40 grams a day. The energy value of the alcohol itself is small, but its use certainly permits of more fat being taken with comfort than would otherwise be the case. The composition of the alcoholic beverages that may be employed in this way is shown in the following table :—

Cc.	Oz.	Alcoholic Beverages.	Alcohol Per Cent. by Weight.	Extractives Per Cent. (as Sugar).	Calories.
20	$\frac{2}{3}$	2 dessertsp. brandy (Cognac) . .	55·9	0·02	78
50	$1\frac{1}{2}$	3 tablesp. gin	30·0	5·50	116
50	$1\frac{1}{2}$	,, rum (Jamaica) . . .	69·6	0·61	245
50	$1\frac{1}{2}$	,, whisky	39·0	...	137
135	$4\frac{1}{2}$	1 glass champagne (dry) . . .	10·4	2·36	112
120	4	,, claret	8·2	2·42	81
120	4	,, French (white) . .	9·5	3·03	95
120	4	,, Moselle or Saar (white) .	7·4	2·31	73
120	4	,, Rhein (white) . .	8·1	2·91	83
30	1	,, sherry	17·5	3·98	42

In some instances the difficulty is best overcome by prescribing fat in an emulsified form. The ordinary commercial cod-liver oils can be used, or an emulsion of olive, or cod-liver, oil may be prepared :—

A

℞ Olive, or cod-liver, oil . 4½ oz.
Cherry laurel water . 3 dr.
Orange flower water . 1 fl. oz.
Carrageen . . . 80 gr.
Essence of bitter almonds 4 min.
Saccharin . . . 2 gr.
Distilled water . . 5 fl. oz.

(Contains 33 per cent. of oil)

B

℞ Castile soap . . . ½ dr.
Cherry laurel water . 5 ,,
Orange flower water . 10 ,,
Saccharin . . . 3 gr.
Essence of peppermint . 6 min.
,, ,, lemon . . 6 ,,
Olive-oil to . . . 1 pint.

(Contains 90 per cent. of oil)

A palatable and efficient emulsion may be made by mixing olive-oil, castor-oil, and glycerine, and adding to this calcium lactate or gum-arabic (4 per cent.) with a little flavouring, such as almond or cinnamon oil, or oil of wintergreen :—

℞ Olive-oil . . . . 3–4 dr.
Castor-oil . . . . ½–1 ,,
Glycerine . . . . 1 ,,
Calcium lactates . . . . 5 gr.
Almond oil . . . . ¼ min.
Chloroform water . . . . to 1 oz.

This emulsion is particularly useful in overcoming the con-
stipation from which most diabetics suffer.

In cases where there is pancreatic insufficiency and the digestion
and absorption of fats are interfered with, solid fats, particularly
those with a high melting-point, should be avoided, as they are
liable to undergo chemical changes in the intestine, with the forma-
tion of irritating by-products, and consequently give rise to dis-
comfort. All fats should be given, as far as possible, in an
emulsified form, since Abelmann has shown that emulsified are
better absorbed than unemulsified fat by dogs whose pancreas has
been partly removed, and Cavazzani found that, while ordinary fat
is rejected, soap is eaten with eagerness by animals after extirpa-
tion of the pancreas. Abelmann obtained the best results with a
natural emulsion, such as milk, 30 per cent. of the fat of large
amounts, and 53 per cent. of small quantities, being absorbed after
complete extirpation, and up to 80 per cent. when portions of the
pancreas had been left behind. The high sugar and protein content
of milk is, as a rule, a drawback to its use in pancreatic insufficiency
associated with diabetes. In some cases of persistent glycosuria,
however, it is well borne, and its addition to the diet does not cause
a marked increase in the glycosuria, or notably affect the excretion
of the acetone bodies—in fact, Donkin has stated that by the use
of an exclusive skimmed milk diet sugar may often be entirely
removed from the urine in a fortnight. This treatment appears to
be most useful in fat, gouty, overfed persons. In other cases even
a few ounces of milk has a bad effect. Various methods of preparing
milk, free from sugar, for the use of diabetics, have been suggested,
but most of these are too complicated, or uncertain, for ordinary use.
" Diabetic, sugarless " milks, put up in sterile bottles, can now be
obtained from several firms, and may be used in moderation, remem-
bering that they contain 10 or 12 per cent. of protein and often $\frac{1}{2}$ per
cent. or more of sugar. As a substitute for milk Williamson has sug-
gested a mixture of washed cream and white of egg, suspended in
water. Three or four tablespoonfuls of fresh cream are thoroughly
mixed with about a pint of water, and allowed to stand for twelve to
twenty-four hours, the fat that has meanwhile floated to the surface
is skimmed off and mixed with water, the white of an egg is added
and the mixture well stirred, on adding a trace of salt and a little
saccharin " a palatable preparation, closely resembling milk, and
practically sugar-free," is obtained. If the functions of the stomach
are being carried out satisfactorily a considerable amount of proteid
may be digested in spite of a deficiency of pancreatic juice, both
in the stomach and upper part of the intestine, where the action
of the gastric secretion will continue, owing to the absence of the

alkaline pancreatic secretion, but even then less than half the albumin of the food is absorbed. Proteids which are digested with difficulty, such as pork, white of boiled egg, &c., must be excluded from the diet. The most useful proteid in cases of pancreatic insufficiency is casein. It may be used in all cases, whether the stomach is functioning normally or not, for it alone among the proteids appears to be broken down, without any preliminary preparation, by the ferment " erepsin " discovered by Cohnheim in the succus entericus. It may be given in the form of milk, or in larger quantities as one of the artificially prepared powders, biscuits, &c., which can be obtained free from sugar. The digestion of both fats and proteins can be assisted by the administration of some active preparation of pancreas two or three hours after each meal, but it is important to make certain that the preparation selected is active and contains a lipolytic ferment, as so many useless pancreatic extracts are now on the market.

Pancreatic and other digestive ferments such as taka-diastase papain, &c., are often useful even when there is no evidence of pancreatic insufficiency, since they prevent the stagnation of food in the intestine with consequent excessive putrefactive changes, and the absorption of toxic products. The latter are probably the primary cause of the glycosuria in some instances, and in others no doubt accentuate the condition. Regular and satisfactory movements of the bowels, with attention to the digestive tract generally, are nearly always points that repay careful attention.

Tea and coffee may be taken in moderation by all diabetics, and form a useful vehicle for the administration of cream. They should, of course, be unsweetened, or be sweetened with saccharin. Lemon-juice with plain aerated water and sweetened with saccharin, or a lemonade made with citric acid (10 grains), glycerine (4 drachms), and water (1 pint) may be taken when desired, but the most generally useful drink is probably Vichy (Célestins) water. This contains chiefly sodium bicarbonate, with smaller quantities of sodium chloride, calcium carbonate, potassium bicarbonate, magnesium carbonate, and sodium sulphate, and is therefore useful in counteracting acidosis. Its effect may be increased by adding sodium bicarbonate (15 to 30 grains), sodium benzoate (4 to 8 grains), and lithium benzoate (4 to 8 grains) to each tumblerful. A large amount of liquid should be avoided, as it increases diuresis. Excessive thirst is most effectually controlled by regulating the diet so that the excretion of sugar in the urine is reduced.

Each case of diabetes is a law unto itself, and can only be satisfactorily treated when all the data obtained by a thorough investigation are available ; but it may be laid down as a general

rule that, since acidosis is the chief danger to be apprehended in severe cases, it is unwise to put the patient on too strict a diet, and that he should not be kept on a carbohydrate-free diet for any length of time. Success depends on the establishment of a tolerance for proteins, and until this has been obtained tolerance for carbohydrates cannot be regained.

The plan of arranging the diet in periods, introduced by von Noorden, is very helpful. By this method the patient is first given a diet containing a small quantity of bread, or other starchy food, the amount varying with the type of case; after two or three days he is put on a low protein diet, consisting chiefly of vegetables, eggs, and fatty foods, such as that shown in the table :—

Vegetable Diet

Grams.	Oz.		Protein.	Fats.	Carbohy.	Calories.
225	8	Coffee	...	...	...	...
40	1½	Cream (2 tablesp.) . . .	1·4	10·0	1·4	100
100	3½	Lettuce	0·9	0·3	2·9	17
25	¾	Onion (¼ large)	0·3	0·4	1·2	10
200	7	Tomato (1 large) . . .	2·4	0·4	8·0	46
22	¾	French ⎰4 tablesp. Ol. oil . dressing ⎱1 „ vinegar . (2 dessertsp.) ⎱¼ teasp. salt, pepper	...	16·0	...	148
50	2	Egg (1 average)	6·6	6·0	...	83
200	7	Spinach (4 hpd. tablesp.) . .	5·4	0·6	6·0	36
15	½	Margarine	0·2	11·8	...	111
249	8	Broth (bouillon)	5·3	0·5	0·5	26
120	4¼	Cauliflower (2 hpd. tablesp.) .	1·1	0·1	0·5	8
15	½	Margarine	0·2	11·8	...	111
56	2	Bacon (weighed uncooked) .	5·6	39·0	...	378
60	2	Brazil nuts (10 large) . .	10·2	40·1	4·2	432
30	1	Lemon juice (2 tablesp.) . .	...	...	3·0	12
225	8	Tea	...	...	...	...
40	1½	Cream (2 tablesp.) . . .	1·4	10·0	1·4	100
56	2	Bacon (weighed uncooked) .	5·9	39·0	...	378
22	¾	French dressing (2 dessertsp.) .	...	16·0	...	148
100	3½	Endive or lettuce . .	1·0	0·2	3·0	16
100	3½	Asparagus (or French beans) .	1·5	0·1	2·8	18
15	½	Margarine	0·2	11·8	...	111
			49·6	214·1	34·9	2309

Carbohydrate . . . . 34·9

Protein sugar $\left(\dfrac{49·6}{6·25} \times 5\right)$ . . 39·7

Total sugar value . . $\overline{74·6}$

This in its turn is replaced in another two or three days by a diet of oatmeal, eggs, and butter or margarine. By repeating this procedure for two or three weeks the acidosis is frequently so much lowered that it ceases to be an immediate danger. The subsequent treatment then depends upon the carbohydrate tolerance of the patient. In some instances marked improvement will result from a week or two on a carbohydrate-free diet, in others the best results are obtained by allowing a certain amount of starchy food, while others again are more benefited by a prolonged period of vegetable or oatmeal feeding. Finally a general diet is worked out on the lines previously indicated for the milder type of case, care being taken, however, to warn the patient that the protein of the food must be strictly limited to the amount shown on his chart.

Whatever diet the patient is eventually put upon, the risk of overtaxing his powers of protein metabolism should be avoided by arranging a period of two or three days on vegetable-fat diet every few weeks, and once or twice a year a longer period of two or three weeks on similar food should be prescribed.

Organo-therapy.—The most serious difficulty in the way of the satisfactory treatment of persistent glycosuria has always been the obscurity of its etiology. When it was proved that excision of the pancreas in animals gives rise to symptoms closely resembling those of human diabetes, and that disease of the gland is found postmortem in some patients who have presented such symptoms during life, it appeared that a solution of the difficulty was at hand. The satisfactory results following the administration of thyroid extract in myxœdema and sporadic cretanism naturally suggested that the use of pancreatic extracts or of the fresh gland might be equally effectual in diabetes, but the results of such treatment have proved most disappointing, and even in those cases of glycosuria where disease of the pancreas was undoubtedly present, the oral administration of fresh or prepared pancreas has, in nearly every instance, failed to produce the hoped-for result. The majority of observers are agreed that, although some improvement of the digestive powers may follow, the glycosuria and other symptoms of the diabetic condition are uninfluenced. In a few instances it has been claimed that some amelioration of the symptoms has been produced. Thus Cowles has reported a case in which an average of over three fresh pancreases were consumed a day, with the result that not only did an improvement in appetite, strength, and weight occur, but the thirst was less, the quantity and specific gravity of the urine fell, and the sugar diminished, although it never quite disappeared. The diet was not restricted much, and nothing but sugar was for-

bidden. Eventually the patient stopped the treatment, the symptoms returned, a large carbuncle developed on the neck, and he died. Post-mortem, the pancreas was found to be represented by a fibrous cord about one-fourth the size of the normal gland. The same treatment was tried with other patients without benefit, but Cowles states that none of these were able to eat such a large quantity of pancreas or to continue the treatment as long. I have met with one case of diabetes in a child of ten, in which a temporary improvement in the general condition and a reduction of the sugar in the urine by nearly half followed the administration of fresh pancreas. Spooner and Pratt state that the limit of assimilation is raised in animals with experimental atrophy of the pancreas by feeding with the fresh gland. In a dog whose pancreas was entirely separated from the duodenum they determined the assimilation limit at frequent intervals for a year, and found that it was never over 35 grams, but after the administration of three sheep's pancreases a day for six weeks the limit of assimilation rose to 80 grams, and continued to rise for a short time after the pancreas feeding was discontinued, the maximum reached being 100 grams.

Arguing that pancreatic preparations given by the mouth are destroyed in the stomach, it has been suggested that they should be administered in capsules that will protect them from the action of the gastric secretions. Some observers, and notably Crofton, have reported satisfactory results, even when no alteration was made in the diet, but it has been generally found that this method gives no better results than the preceding. I have systematically given various pancreatic extracts that laboratory experiment has shown to contain active ferments, in various ways by the mouth, and although the digestion and general condition of the patient has frequently shown a marked improvement, especially when there has been evidence of pancreatic insufficiency, I have not found that the glycosuria or secondary disturbances of metabolism were favourably influenced.

Subcutaneous injection has been resorted to by some observers, with a view to avoiding destruction of the ferments contained in the extract by digestion in the alimentary tract, but with equally unsatisfactory results. According to Leschke, fresh extracts of pancreas are not only useless, but increase the elimination of sugar in diabetic persons and animals, and also induce glycosuria in normal animals, with a toxic and often fatal result. Basing his procedure on experiments which suggested that the hormone of the pancreas is antagonistic in its action to the hormone of the supra-renals, and that in pancreatic diabetes the lack of the pancreatic secretion and the predominance of the internal secretion of the supra-renals

explains the glycosuria, Zuelzer and his associates have attempted to treat diabetes with expressed extracts of the pancreas taken at the height of digestion and rendered less toxic by removing the albumens with alcohol. Satisfactory results were reported in several cases, the urine being freed from acetone and aceto-acetic acid and the sugar much reduced in amount. Employing the same method, Forschbach was able to confirm Zuelzer's conclusions with depancreatised dogs, but with human diabetics found that although the sugar excretion was temporarily diminished, the acetone bodies were not affected, the temperature was raised, and the patient became acutely ill. He is inclined to attribute the diminished glycosuria to the accompanying fever, and considers that the pancreatic hormone is too dangerous for practical employment in diabetes. With the idea of restoring the balance between the internal secretions of the pancreas and supra-renals, and working on the principle by which exophthalmic goitre is treated with the serum or milk of thyroidectomised animals, Brück has suggested that the missing neutralising pancreatic secretion might be supplied to diabetics by administering the milk or serum of animals in which adrenalin has been excluded from the circulation, but I am not aware that his suggestion has been carried out in practice.

When considering experimental diabetes, we saw that Minkowski proved that if a portion of the pancreas were implanted in the subcutaneous tissue of the abdominal wall, it sufficed to prevent glycosuria when the remainder of the gland was removed. In 1908 he published a full report of the effects of grafting pancreatic tissue in dogs, and conclusively proved that if the graft secures a sufficient blood supply it grows and functions to such an extent that the animal's own pancreas can be completely removed without the occurrence of diabetes. It would seem that this procedure presents great therapeutic possibilities, but I can only find records of two cases in which treatment on these lines has been attempted. One was reported by Watson Williams and the other by J. W. Allan. In the former case a sheep's pancreas was grafted into a patient suffering from severe diabetes, but without any result, death taking place a fortnight after the operation from diabetic coma. Post-mortem, the pancreas was found to be " large and apparently quite healthy to the naked eye." In the other case a cat's pancreas was used, but in this too failure resulted, the pancreas dying and sloughing out. It may be pointed out that in both these cases the operation was not attempted until a late stage, and Minkowski has shown that in animals the transplantation must be undertaken before diabetes has been induced, as otherwise the graft does not grow and the wound fails to heal.

If the view that the islands of Langerhans are the source of the internal secretion of the pancreas is correct, it would seem probable that an extract prepared from them would have more effect than an extract of the whole gland. With this object Rennie and Fraser treated five diabetics with a preparation of the macroscopic chief islands found in certain fishes, and state that some improvement followed its use. Their results have not, however, been so far confirmed, and the authors themselves have not published any additional cases.

The observation of Hédon that the normal pancreas only checks glycosuria when it is so placed that its internal secretion enters the portal circulation directly, offers a reasonable explanation of the almost uniformly negative results of the various attempts that have been made to treat diabetes with preparations of pancreas, and appears to make the realisation of a specific treatment along these lines more difficult than ever. It would seem, however, that there is a group of cases in which the administration of pancreas by the mouth materially improves the condition of the patient, but as a rule there is in these some interference with the flow of pancreatic juice into the intestine.

Basing their treatment on the effects of secretin as a stimulant of the pancreas, Moore, Edie, and Abram employed an acid extract of duodenal mucous membrane in diabetes. They found that when this was given by the mouth, the sugar in the urine gradually diminished in some cases and finally disappeared in a few. In others, although there was an improvement in the digestion, no effect on the sugar output was produced. Bainbridge and Beddard, however, noticed no amelioration of the symptoms in the cases that they treated by this method, and suggested that any improvement that takes place is to be attributed to the diet and not to the secretin. Bainbridge also found that the yield of secretin from the duodenal mucous membrane is almost or quite as great in diabetic as in non-diabetic people, and attributes the failure of some other observers to find it to its destruction from rapid post-mortem changes. J. R. Charles, N. B. Foster, and other observers state that in their experience the administration of secretin does not affect the glycosuria. Even if it could be shown that the glycosuria could be controlled by this means in some cases, it is open to question whether the treatment might not in the long run do more harm than good, for the excessive artificial stimulation of the diseased tissue that may remain in pancreatic diabetes, although it may at first induce increased activity, is likely to eventually bring about fatigue and cause more rapid degeneration than would have occurred if it had been left alone. The intravenous injection of secretin has been

suggested, but it has been shown by Starling that it gives rise to acute inflammation of the intestine, and even to gastric ulcers in animals, as the pancreatic juice is not met and neutralised by the acid gastric contents which normally cause the flow. This objection does not apply to its administration by the mouth, as the resulting secretion is then gradual, and corresponds to the acidity of the gastric juice reaching the intestine.

Cohnheim's work upon the effects of a mixture of pancreatic and muscle extracts in glycolysis has suggested the use of such a mixture in diabetes, and it has been employed for this purpose by Crofton, Sewall, and others.

When considering the alleged favourable results obtained with any form of treatment in diabetes, it is important to bear in mind that the clinical course of the condition is at times subject to considerable variation under a great variety of conditions, and that favourable results have been claimed to be produced by a number of remedies that have probably no specific relation to the pathological processes present. The effects of alterations in the general hygiene, surroundings, and diet of the patient have also to be allowed for.

Drugs.—The drugs that have been employed at various times in the treatment of diabetes are exceedingly numerous, but only very few are generally acknowledged to be of service. Even these are not curative, and improve the condition of the patient most when they are employed in conjunction with careful restriction of the diet.

Opium and its derivatives, morphine and codeine, are not infrequently employed, and have stood the test of time. It is found that as a rule they control the glycosuria and polyuria more effectually than any other drug when they are given in sufficiently large doses. In some cases however, opium, like all other remedies, is useless.

Codeine is probably the opium preparation most frequently prescribed in persistent glycosuria, since it does not tend to make the patient sleepy, nor does it affect the bowels as readily as opium or morphine. At first it should be given in small doses, a quarter to half a grain three times a day, and then be gradually increased until the glycosuria is controlled or the patient's limit of tolerance for the drug is reached. Often 12 grains or so may be given in three doses during the day without producing any untoward result, but in many instances 6 or 8 grains may cause the patient to lose ground and give rise to diarrhœa. Codeine may be administered in pill form combined with cascara sagrada :—

℞ Codeinæ . . . . . gr. $\frac{1}{4}$ to $\frac{1}{2}$
   Ext. Casc. Cas. . . . gr. 2
   Pulv. Glyc. . . . . gr. 2
   Ext. Gent. . . . . q.s.

or with cascara and nux vomica—

℞ Codeinæ . . . . gr. $\frac{1}{4}$ to $\frac{1}{2}$
   Ext. Nuc. Vom. . . . gr. $\frac{1}{2}$
   Ext. Casc. Sag. . . . gr. $\frac{1}{2}$

Codeine is, however, considered by some authorities to be inferior in its effects on sugar excretion to opium and morphine, and there can be no doubt that these sometimes succeed when codeine fails ; moreover, as it quickly loses its first sedative effect, it has often to be replaced by other drugs after a short interval.

Morphine is much less expensive than codeine. According to Mitchell Bruce, acetate of morphia is the most useful salt, and acts better when given by the mouth than when injected subcutaneously. A small dose, one-sixth of a grain or so, three times a day, is sufficient to commence with. The dose is gradually increased until one grain, or even more, is being taken three times in the twenty-four hours.

Opium is believed by many observers to give more uniformly satisfactory results than either of its alkaloids. Starting with half a grain or its equivalent (e.g. Pil Saponis Co., gr. 2 to 4), three times a day, the dose is gradually increased until 12 or 15 grains are being taken daily. Some prefer the watery extract of opium, which can be given in much the same doses as codeia.

According to Ralfe, opium has the greatest effect in restraining diuresis when taken about one hour after meals, and is also then less likely to cause dyspeptic symptoms or derange the stomach. Although diabetics are very tolerant of opium and its alkaloids, so that large quantities can be taken without producing bad results, they often exhibit individual peculiarities with regard to its different preparations,. Opium for instance, in the form of Pil Saponis Co., can sometimes be taken without discomfort, when morphine or codeine will give rise to headache and giddiness. Occasionally the best effects are obtained by combining crude opium with one or other of its alkaloids. In fixing the dose it must be remembered that this will vary with different cases. As a rule the administration should be pushed until the glycosuria is controlled, or no further reduction in the sugar excretion and volume of urine follows an increase in the dose ; when this point is reached the dose should be maintained at that level. Its effects should always be carefully watched, particularly with regard to the production of constipation and dyspeptic symptoms. When these cannot be otherwise

controlled, its administration must be discontinued. Since constipation is often associated with severe acidosis and threatening coma, opium must be used with great care in such cases. Landergren states, however, that in certain cases opium markedly reduces the amount of acetone and oxybutyric acid in the urine, and claims that in threatening coma opium may often prove of value by diminishing the acidosis. When nephritis is present opium should be avoided, or be given with very great caution. Opium, like its alkaloids, gradually loses its effect, although not so rapidly as a rule.

The way in which opium and its alkaloids act in diabetes is not definitely understood. According to Lépine it produces an effect on the liver, through the nervous system, preventing the excessive production of sugar. Von Mering and Minkowski suggest that it inhibits the formation of sugar from albumen. Roberts considers that it diminishes the appetite, and so less sugar is excreted in the urine. Others hold that it reduces the level of metabolism generally. In view of the theories that are at present held with regard to the influence of the ductless glands on carbohydrate metabolism and their relation to the nervous system, its effect as a nervine sedative, and its known action on vaso-motor system and on glandular secretion, offer the most likely explanation of the beneficial results that follow its use in most cases.

Belladonna, either alone or in combination with opium, appears to be useful in some cases, but owing to the dryness of the throat, to which it gives rise, it cannot usually be tolerated for any length of time. Rudisch has strongly recommended the use of atropin methylbromide in diabetes, and states that when given in fairly large doses in conjunction with dietetic treatment it greatly aids in lessening or preventing glycosuria. Forscheimer found that the same results were obtained whether atropine methylbromide, atropine sulphate, or belladonna was used, and that not only did the glycosuria diminish in many cases, but that the excretion of acetone bodies also lessened and carbohydrate tolerance was increased. According to this author belladonna is more particularly adapted to mild cases, and does little good in the severe types. In the hands of other observers this method of treatment has not been so satisfactory. Mosenthal, for instance, found that atropine sulphate effects no change in carbohydrate tolerance of sufficient importance to make the drug of clinical value in the treatment of diabetes. Experimenting with depancreatised dogs, Wallace found that the drug had no effect on sugar excretion.

Pilocarpine injections have been stated to cause a diminution in the amount of urine and sugar excreted in some cases, but in others they have been found to do no good.

Antipyrin was suggested as a remedy in diabetes by Gönner, and, according to Dujardin-Beaumetz, in doses of 30 to 60 grains daily it diminishes the quantity of urine without there being any increase in the percentage of sugar. Its action is, however, only temporary, and, as it is liable to give rise to digestive disturbances and albuminuria, it should not be used for long. *Pyramidon* is said to be less likely to upset the stomach, although it has not such a marked effect on the urine as antipyrin. Many observers have found both drugs useless, but as there appear to be marked idiosyncrasies, and the results vary very much with different patients, they are worth a trial when other methods of treatment fail.

Bromides.—In 1866 Begbie employed potassium bromide in the treatment of two cases of diabetes with success. Since then it has been used by other physicians, and has been recommended by v. Noorden, Osler, and Saundby in cases where there is great nervous irritability or excitement. It does not appear to have as marked an effect in such cases as might be expected, and, owing to its depressing action in many cases, its use has frequently to be discontinued. Ammonium, or lithium, bromide may be substituted, and do not so readily give rise to depression. *Valerian,* which was recommended by Trousseau for polyuria and is a nerve stimulant, may be given, in the form of the ammoniated tincture, to counteract the depressing effect of the bromides.

Bromides, antipyrin, and other drugs which depress the nervous system are to be avoided in cases where the patellar reflex is absent or weak.

Arsenic.—Experiments on animals have shown that the administration of arsenic in sufficient doses, for an adequate time, causes the glycogen to disappear from the liver, and that puncture of the floor of the fourth ventricle will not then give rise to glycosuria. Toxic effects must, however, be produced, and ordinary medicinal doses are of no use. Varying results have been reported from the use of arsenic in clinical work on diabetes. Some physicians have stated that, after the excretion of sugar has been reduced by diet and opium, arsenic will effect a cure, while others have found that, although it is useful in some mild cases, it has no effect in the severer forms. Forscheimer, on the other hand, states that it is especially indicated in severe cases, but should always be combined with diet. He considers that it is also useful in neurotic, debilitated subjects. He points out that to get the best results mild toxic effects must be produced. Gradually ascending doses are given until this result is obtained, and the dosage is then gradually reduced. Arsenic used in this manner is said to diminish the glycosuria and acetonæmia, but does not increase sugar tolerance. Unlike

opium the effect is not lessened by prolonged administration, but when the drug is discontinued a gradual falling off is observed. A repeated course of the treatment, however, again controls the glycosuria and excretion of acetone bodies. Arsenic has been usually given in the form of liquor arsenicalis in diabetes, but arsenite of bromine (Clemens' solution), in doses of 3 to 5 minims once or twice a day after meals, has also been employed, and combinations of lithium and sodium arsenate in pill form, or dissolved in aerated water, have been used, especially in gouty cases. Dujardin-Beaumetz recommends 5 grains of lithium carbonate and 2 minims of liquor arsenicalis in a glass of Vichy, or other alkaline, water. Le Gendre advises five or six pills a day, according to the toleration, of the following composition :—

$$\text{R} \quad \begin{array}{llll} \text{Strychnine sulphate} & . & . & . & \text{gr. } \frac{1}{120} \\ \text{Sodium arsenate} \ . & . & . & . & ,, \quad \frac{1}{60} \\ \text{Codeine} \quad . \quad . & . & . & . & ,, \quad \frac{1}{6} \\ \text{Quinine valerinate} & . & . & . & ,, \quad \frac{3}{4} \\ \text{Extract of valerian} & . & . & . & q.s. \end{array}$$

in cases where there is depression of the nervous system, and the patellar reflex is absent or weak.

Quinine.—According to Lépine, quinine has an anti-glycogenic action, and is of use in diabetes for this reason. Although some observers have stated that it diminishes the glycosuria, it is generally held that it merely acts as a general tonic, and is therefore only useful in cases where a stimulant line of treatment is indicated. It may also be of service when the symptoms of diabetes have followed attacks of malaria, or the patient has resided in a malarious neighbourhood.

Anti-syphilitic Treatment.—In a small proportion of diabetics the condition appears to be due to pathological changes set up in the pancreas, and possibly also in the nervous system, by syphilis. Feinburg and v. Noorden have recorded such cases, and found that improvement was frequently brought about by treatment with mercury and potassium iodide. I have had two patients under my care in which persistent glycosuria followed infection with syphilis, and in both a course of anti-syphilitic treatment much improved the general condition and reduced the glycosuria, although the urine was not rendered sugar-free in either. As mercurial stomatitis and intestinal catarrh are very liable to develop in diabetics, the patient should be constantly watched, and the treatment be carefully regulated. Von Noorden states that in several of his cases fatal complications, including gangrene of the foot, hæmoptysis, and rapidly progressing tuberculosis, occurred during the mercurial course.

Iodide of potassium has been tried as a remedy in cases of diabetes not of syphilitic origin, but Dickinson, as the result of a series of careful observations, came to the conclusion that any diminution in the excretion of sugar that occurs is due to loss of appetite and general depression of function, and not to real mitigation of the disease.

Iodoform was found by Moleschott and Frerichs to cause a temporary improvement in some cases, but other observers have stated that no good effect followed its use.

Uranium nitrate has been recommended by Hughes, and later by West, who state that it caused a marked diminution in the excretion sugar, and also diminished the thirst and amount of urine in the cases where they tried it. By starting with small doses of 1 or 2 grains, freely diluted with water, twice a day after meals, the danger of digestive disturbances and albuminuria is avoided. The dose is gradually increased every few days until the desired effect on the urine is produced. In some cases as much as 15 to 20 grains have ultimately been given three times a day.

Intestinal Antiseptics.—At one time diabetes was thought by some observers, to be a specific infectious disease and attempts were made to reproduce the condition in dogs by feeding them with fæcal material from diabetic patients, but without result. According to Herter, glycosuria has been produced in cats and dogs by intravenous injections and by feeding experiments with bacteria isolated from diabetic stools, but the data are so scanty that the evidence cannot be regarded as conclusive. Spontaneous diabetes mellitus has been described in dogs by Friedberger, Frönner, Schnidelka and Eichorn, and others. Some five years ago I isolated a sugar, having the reactions of dextrose and yielding dextrosazone crystals, from the urine of a pet dog whose mistress was suffering from diabetes. The condition has also been met with in horses by Heim, Rueff, and Dieckerhoff, and in monkeys by Leblanc. Cases of apparently infectious origin have been reported by several observers. Teissier, at the first French Congress of Medicine, reported the case of a washerwoman aged sixty-two who became glycosuric after having washed for six months the linen of a diabetic, and her little girl also became affected with the disease. There is, too, the case of a gouty patient whose mother had been diabetic for twenty years, and who developed the disease in his turn ; six months later the cook, who had washed the handkerchiefs of her master, became glycosuric ; and finally, the disease showed itself in a woman of fifty employed by the family for ten years to mend clothes. A coachman became glycosuric shortly after his master, and a restaurant-keeper who took his meals at the same table with a

diabetic sister-in-law became diabetic at the end of six years. Kuelz has reported a case of conjugal diabetes in which four persons occupying the same house became diabetic, and Naunyn observed five cases of diabetes under the same roof. Senator, at a meeting of the Berlin Medical Society in 1908, quoted the case of a doctor aged forty-two who became diabetic after amputating the thigh of a diabetic patient. He discovered that there were four cases of diabetes in the immediate neighbourhood, all in the same street, and all taking their meals at the same restaurant, which was kept by a glycosuric.

The most striking evidence in favour of an infectious origin in glycosuria is furnished by the occurrence of diabetes in husband and wife (" Conjugal Diabetes "). The possibility of this occurrence has been supported by Debove, in France, who, out of fifty cases of diabetes, found five in which husband and wife were both affected, and similar cases have been recorded by Dreyfous, Gaucher, Labbé, Rendu, and Schmitz. The last had a very large practice at Neuenahr, in which he had seen an immense number of cases of diabetes, and was certainly inclined to believe in the possibility of transmission. Senator, in the discussion referred to, stated that he had collected the histories of 516 married pairs, in which either husband or wife was diabetic, and in eighteen cases the second partner had become diabetic, giving a proportion of 3·5 per cent. cases of conjugal diabetes. Although we have no data by which we can fix accurately the period of incubation of diabetes, supposing it to be an infective disease, yet as we know that traumatic diabetes develops in at least six months after the injury, and usually in less time, he therefore accepts that period, and excludes from his list all those marriages which had lasted for only six months or less ; in this way the percentage of cases rose to 3·7, while by eliminating other seventy-four couples whose marriages dated from less than a year, the percentage rose to 4·1. He admits that it would be right to exclude all cases with distinct hereditary predisposition ; but taking the figures as they are the proportion is too feeble to support the theory were it not that there are other facts in its favour. In the course of the discussion Neumann of Potsdam said that during the previous five years, with the aid of his colleagues and the chemists of the town, he had collected 180 cases of diabetes from a population of 59,881 ; among these he found only three instances in which both married partners were affected, and two of these should be excluded, as in one case the wife was pentosuric only, and in the other the husband became diabetic three years after the death of his wife. In the single remaining case the wife was diabetic and suffered from Graves' disease, and the husband developed the disease after an accident.

to her. Albu said he had seen no case to justify the view that diabetes was contagious, but he knew of two instances illustrating the fallacies surrounding such apparent cases ; in one the wife became diabetic after her husband, and at the time Albu knew of no hereditary tendency on her side, but some years after he treated her nephew for diabetes and obtained unquestionable evidence of the family predisposition. In the other case the glycosuria developed by the second of the married pair turned out to depend upon cancer of the pancreas. Finally, Ewald stated he had met with no case of conjugal diabetes, and, considering that the statistics presented ranged from 1 to 20 per cent., he thought the entire relation was accidental. In all such statistical inquiries the influence of heredity must be borne in mind. In Germany Jews are numerous, and amongst them diabetes is so common that few families escape it altogether, and as they only marry with their own people, among married Jews suffering from diabetes there should be a distinct proportion of cases in which both partners show the disease. On the other hand, races not predisposed to diabetes, among whom the annual mortality from it does not exceed 6 or 7 per 100,000 of population, the probability of both members of a married pair developing the disease becomes infinitely small, and it is in accordance with this view that so-called conjugal diabetes is very rarely met with in this country.

Although a specific infectious theory for all cases of diabetes has now been abandoned by nearly all observers, there are many who consider that in some instances, and particularly where disease of the pancreas is the cause of the condition, an abnormal intestinal flora may be the origin of the mischief. The evidence in favour of this view is at present rather clinical and inferential than experimental, but it is not inconsistent with modern views with regard to diabetes, and furnishes a basis for a preventive, if not for a curative treatment. Certain drugs which have been employed in the treatment of diabetes with satisfactory results are intestinal antiseptics, and it is probable that their effect is largely due to this fact.

Sodium salicylate has been occasionally used for twenty years or more. Some observers, notably Ebstein, have reported a reduction in the excretion of sugar, and a general improvement in the condition of patients to whom it had been administered. Ebstein considers that it is chiefly useful in recent cases, and advises that it should be given in large doses 75 to 150 grains in the twenty-four hours. Brunton and Ralfe state that it is chiefly useful in gouty glycosuria. Salicylate of bismuth, in the form of $7\frac{1}{2}$ grain powders twice a day, has been employed by Schmitz with good

results. Other observers have employed phenyl salicylate (salol), or acetyl salicylic acid (aspirin). These drugs are all recognised as more or less efficient intestinal antiseptics, and, according to Crow, salicylates are excreted in the pancreatic juice and bile after absorption. Salicylates are also said to stimulate the activity of the thyroid. When giving these drugs, or any other salicylate compound, it must be remembered that the urine will give a purple coloration with perchloride of iron, which is liable to be mistaken for the similar reaction with aceto-acetic acid, the latter is, however, diminished after the urine has been boiled for some minutes, whereas the salicylate reaction persists unchanged. As most salicylate compounds have a tendency to produce constipation, the condition of the bowels should be watched during their administration.

Hexamethylenamine (urotropine) is one of the most generally useful antiseptic drugs for internal administration that we possess. The experimental investigations of Crow have demonstrated that it is absorbed into the circulation, and can be found in the blood for twenty-four hours after its administration by the mouth. It is excreted in the urine, by all the mucous surfaces, in the bile, synovial fluid, pancreatic juice, saliva, plural and cerebro-spinal fluids, &c. &c. A dose of 75 grams a day will prevent bacterial growth in the bile and pancreatic ducts. It is therefore exceedingly useful in controlling infections of these and other regions, and it is possible that to this its effect in diabetes is due. Forchheimer used it in 5 grain doses in the treatment of diabetes, and found that the glycosuria was, in many instances, reduced, and the carbohydrate tolerance improved. He considers that it is especially indicated in cases where dietetic regulation is impossible, but does not recommend it in severe cases. It is to be noted that after the administration of this drug the urine may reduce copper solutions, but it does not generally reduce bismuth, and never ferments with yeast.

Yeast is a very old remedy for diabetes. Caessaet and Williamson both report good results from its use. The latter gives it in doses of two dessertspoonfuls in water, but points out that it must be fresh and free from liquid, or severe diarrhœa may be produced. Any improvement that follows is, however, only temporary.

Castor-oil, Carlsbad salts, and other laxatives are frequently required in the treatment of diabetes. Constipation is one of the difficulties that calls for most constant attention, and if the bowels are carefully regulated it will be found that most forms of treatment prove more satisfactory.

The Massive Saline, or Fasting-purgation (Guelpa) Treatment. — Taking the view that diabetes is an auto-intoxication

of intestinal origin, Guelpa advises free purgation, combined with complete abstinence from food for two, three, or even more days. The one without the other, he maintains, is harmful, purgation without complete rest to the digestive tract causing as much constitutional disturbance as abstention from food without daily and abundant evacuation of the intestinal contents. The somewhat paradoxical statement is made that the longer starvation is continued by this method, the less is the feeling of hunger ; for the first twenty-four hours there is some discomfort from the interruption of the usual habits, but by the second or third days this is much less marked. It is also said that the more abundant the purge, within limits, the less is the colic produced. If, for example, a single glass of Hunyadi Janos water is drunk several watery stools result, and there is discomfort for some hours, but if a whole bottle is rapidly taken, say, within fifteen minutes, no colic whatever occurs, and a satisfactory and complete action follows within a short time, sometimes in a few minutes. The purge must be dilute, and be taken hot. To apply these principles to the treatment of diabetes the patient is given a whole bottleful of warm Hunyadi Janos, or other dilute saline solution, each evening for three days, with complete abstinence from all nourishment except water, weak tea with milk, clear strained vegetable soup, or any hot infusion, according to the degree of thirst complained of. He is then put upon a diet of green vegetables, beef-tea, or meat extracts (20 oz.), milk (20 oz.), well diluted with water or tea, for another three days. It is said that in many cases the sugar disappears entirely from the urine, acetonæmia is controlled, and a marked improvement in the general symptoms occurs. If the resumption of an ordinary diet causes a return of the sugar, the amount excreted is always much less, and can be again reduced by a further course of treatment—in fact, it is often advisable that three or four periods of fasting and purgation, each not exceeding three days, and extending over six weeks, should be arranged for at the outset. Satisfactory results have been reported with the Guelpa treatment by R. W. Philip, A. A. Warden, Oscar Jennings, and a few others, but I have never been able to persuade any of my patients to attempt it.

Vaccines.—If the view that some cases of diabetes are dependent upon bacterial infection and toxæmia is correct, it might be expected that treatment with vaccines would be useful. As it is impossible to make cultures directly from the pancreatic ducts in cases where the pancreas is probably at fault, the only way is to prepare them from the fæces, testing the patient's blood against the cultures so made by the opsonic and agglutination tests. I have carried out this method of treatment in three cases of persistent glycosuria

where there were probably floating gall-stone in the common bile
duct, and which, for various reasons, had not been submitted to
operation. In two the results were for a time satisfactory, the
glycosuria and other symptoms diminishing ; but in the third no
effect was produced, possibly because the causal organism had not
been used in the preparation of the vaccine.

Santonin has been said to reduce glycosuria, but an exhaustive
trial by Walter Löfer proved that it had little or no effect on sugar
excretion in the cases in which he used it.

Jambul.—The seeds of *Eugenia Jambolana* (*Syzygium Jambol-
anum*) are much used in the East in the treatment of diabetes, and
although some observers in Europe have reported satisfactory
results, they have proved quite useless in the experience of many.
The seeds are given in doses of 5 to 30 grains in the form of
powders, cachet, or pills, or as the liquid extract, in doses of $\frac{1}{2}$ to 2
drams. According to Colostoni and Martz, the fresh seeds contain a
substance that hinders the formation of sugar from starch, and it
may be that the varying effects observed, especially in Europe, are
due to changes in this substance, or some other constituent of the
seeds, on keeping.

Taka-diastase.—Beardsley has reported great relief from the
symptoms of diabetes in several cases following the administration
of taka-diastase, in 5 grain capsules after each meal and at bedtime.
In one instance the sugar disappeared completely, while in others
it diminished in amount ; the polyuria was relieved, and the patient
gained in weight. Improvement was found to cease when the drug
was stopped, but was continued when it was resumed. No unto-
ward effect was traceable to its prolonged administration.

Alkalies.—Large doses of alkalies are said to interfere with
glycogenesis, as the glycogenic ferments do not act well in an
alkaline medium (Lépine). It is not likely, however, that the good
effects that follow the usual therapeutic doses given in diabetes
are due to this. They undoubtedly tend to counteract the acidosis
from which all diabetics are liable to suffer, but many cases in which
there is no evidence in the urine of excessive acid formation are also
benefited by the administration of alkalies. The drug most com-
monly selected is bicarbonate of sodium, but the carbonate, or
acetate, of sodium, the bicarbonate, carbonate, tartrate, citrate,
or acetate of potassium, and carbonate of ammonium, or lithium,
are also used sometimes, the lithium salt being particularly useful
in gouty patients. The drug may be given in simple solution, or
the patient may be sent to a Spa, such as Carlsbad, where he gets
the carbonate of soda along with a sulphate, or to Vichy, where he
gets the carbonate without the sulphate. Poques is recommended

in asthenic cases, and Vittel for gouty diabetes. Richardiere advises an alkaline course, of 60 to 150 grains of sodium bicarbonate in twenty-four hours, for two or three weeks, every three or four months, and states that it is most useful in mild cases. Alkalies are especially indicated when an anti-diabetic regimen has not fully succeeded. Alkalies should be avoided as far as possible in cachectic cases, when pulmonary tuberculosis is present, and in advanced stages of the disease where there is wasting, unless marked acidosis and symptoms of threatening coma occur. In cases where there is gastritis and hyperchlorhydria the carbon dioxide set free in the stomach, when sodium bicarbonate is given, may cause serious discomfort, and in such cases it is advisable to substitute magnesium hydroxide or calcium salts. The latter have been found by some observers to influence the sugar excretion to a more marked degree than sodium bicarbonate, and, since calcium salts are known to be of use in many toxic conditions, it is not unlikely that their effect in diabetes may be partly dependent on some such action. Willis in 1679 described a case of diabetes treated with lime-salts that recovered. In 1895 Grübe revived the treatment with calcium salts, and insisted on its value. Glycero-phosphate of calcium and magnesium have also been recommended by Robin, with the object of making good the loss of calcium and magnesium salts in the urine that occurs in diabetes. The beneficial results that follow a course of treatment with Bethesda water, containing chiefly bicarbonate of lime and magnesia, and Contrexeville, sulphate and bicarbonate of lime with some sodium sulphate, may possibly be dependent upon the presence of calcium salts.

Lactic Acid.—According to Cantini, pure lactic acid, given in water directly after meals, aids the digestion of nitrogenous food, and allows a strictly nitrogenous diet to be taken for a longer period without the occurrence of gastro-intestinal disturbances than would otherwise be the case. It is given in the form of a lemonade, 5, 15, or 20 grams being mixed with a litre of water, and flavoured with peppermint or anise. Half a wineglassful of this mixture, with half a gram of bicarbonate of soda added, may be taken after food, and every hour or two.

Other drugs that have from time to time been prescribed and advocated by various observers are so numerous that a mere list of them would occupy a large space. In addition to the above may be mentioned chloral, sulphonal, phosphorus, phosphoric acid, strychnine, cocaine, iodine, picric acid, benzoic acid, hydrogen peroxide, valerian, guiacol, camphor, calcium sulphide, creosote, ergot, benzosol, methylene blue, pepsin, rennet, oxygen inhalations, &c.

Electricity has been frequently tried in the treatment of diabetes, but without any striking benefit. It has been recently stated that high frequency currents diminish the glycosuria experimentally produced in animals, but I am not aware that they have been used for this purpose in human diabetes.

The very varying success that results from the employment of such a variety of drugs, and other forms of treatment, in persistent glycosuria renders it quite plain that no one is specific, and suggests that different lines of treatment are required for different cases. This is what might be expected if we accept the view that diabetes is not *a disease*, but a series of symptoms that may arise from a variety of causes. Some remedies, such as opium and its alkaloids, alkalies, &c., are undoubtedly more frequently useful than others, but even these cannot be employed in every case with the certainty that a cure, or even an amelioration of the symptoms, will result.

General Management and Hygienic Treatment.—As mental shock, *worry, and excitement* undoubtedly play a part in the production of glycosuria in some cases, and tend to intensify it when present in others, it is most important that the patient should be placed in congenial *surroundings* and under conditions that will soothe his nervous system. A cheerful state of mind, freedom from business and other worries, and a full confidence that the treatment he is undergoing will benefit him, are material factors in dealing with every case. A fair amount of *exercise*, taking care to avoid over-fatigue, is advisable in most cases, but in those where there is marked wasting, muscular exercise is likely to increase the glycosuria and acetonæmia, and cause depression. *Massage* is sometimes a useful adjuvant to other forms of treatment, and more particularly in diabetes associated with arterio-sclerosis, in obese patients, and those who are too weak to take muscular exercise. Regulated Swedish gymnastics may also be prescribed in suitable cases. Open-air exercise is, however, to be preferred, if possible, and most cases of chronic glycosuria do best when they are able to obtain an abundance of *fresh air and sunshine*—in fact, some authors contend that open-air treatment is as beneficial in diabetes as it is in tuberculosis. The *clothing* should be warm, and in winter woollen, or silk, garments should be worn next the skin. The action of the skin should be promoted by warm *baths* followed by friction, especially if the surface of the body is dry, but sea-bathing and cold plunges should not be indulged in.

A *warm climate* is often recommended, and since a low external temperature means that more energy must be produced within the organism to keep up its internal temperature, a warm, sunny

place would seem advisable. But as the bodily and mental fatigue incident on a long journey are often harmful, and in serious cases may precipitate diabetic coma, the slight advantage that would result from a change of environment is frequently more than counter-balanced by the risks involved. The same objection also applies to the *Spa treatment* of diabetes. A visit to Carlsbad, Marienbad, Vichy, or Neuenahr is often very useful in mild cases, especially those who are obese or gouty, for the patient is removed from his usual routine, and lives a quiet, regular, and peaceful life ; he is in the open air a great part of the day, he takes suitable bodily exercise, and his diet is carefully regulated ; but the long journey involved, and possibly the worry as to ways and means, may sometimes counteract any good that would otherwise result, particularly in the severer type of case. One is often asked whether the use of *tobacco* should be given up ? Although acute nicotine poisoning is known to give rise to glycosuria, owing apparently to its action on the supra-renals, it is not probable that smoking in moderation aggravates the condition to any appreciable extent. The abuse of tobacco is, however, undoubtedly dangerous, especially when the heart is not quite sound. *Women* who suffer from diabetes are not uncommonly sterile, but it is not safe on this account to allow them to marry, for should pregnancy occur it is likely to seriously aggravate the condition, and may rapidly bring about a fatal termination. Abortion frequently occurs, and pregnant diabetic women are very likely to develop tuberculosis, 20 to 25 per cent. of cases, according to Neumann, suffering in this way. When a married woman is found to have diabetes it is necessary to instruct her to avoid conception. Should she become pregnant anti-diabetic treatment should be instituted with great care, all drugs being avoided as far as possible in the interests of the fœtus. If dietetic and hygienic measures do not suffice to control the condition, it will probably be necessary to induce abortion. Should this be avoided in the early stages it may become necessary in the last three months in the interests of both mother and child, for a strict anti-diabetic diet is not favourable to healthy development, and the fœtus is liable to grow abnormally large, causing difficulty in delivery at term. When labour has commenced it should be terminated as speedily as possible, whether the child is living or not, but no anæsthetic should be given. After delivery the most careful antiseptic precautions should be observed, and even the smallest tear of the perineum, or soft parts, be carefully attended to. The condition of the urine, and the general state of the patient, must be assiduously watched for evidences of severe acidosis, &c., as the disease often makes very rapid progress during the lying-in period.

Experience has shown that even in healthy women sugar tolerance is lowered at this time. Hirschfeld found that glycosuria occurred in 10 per cent. of healthy pregnant women after 100 grams of dextrose. If glycosuria only appears late in pregnancy, the sugar excretion does not exceed 20 grams or so a day, and is not accompanied by other symptoms ; it is probable that it is transitory and will spontaneously disappear after delivery. The patient should, however, be very carefully watched.

Symptoms of diabetes sometimes develop in men shortly after marriage, and it is possible that *sexual excitement* may occasionally be the determining factor. I have had one case under my care in which sugar appeared in the urine six months after marriage in a hæmophiliac patient, but was quickly controlled by diet and rest in a nursing-home. It is probably advisable that males suffering from diabetes, especially the more severe forms, should not marry, but each case must be judged on its merits, and, in any event, excessive excitement should be studiously avoided.

Treatment of Complications.—*Constipation* is the complication which is most commonly present, and has most frequently to be guarded against in diabetes. It is best treated with saline purgatives, mineral waters, senna, or castor-oil. Drastic purgatives should be avoided.

Inflammation of the Gums.—The teeth should be regularly cleaned, and a mouth-wash consisting of listerine or a solution of boracic acid (1 dram), borax (2 drams), and pot. chlor. (1 dram) in camphor water (1 pint), or a 3 per cent. solution of sodium bicarbonate should be used. A mixture of borax (2 drams), boracic acid (1 dram), tinct. myrrhæ ($\frac{1}{2}$ oz.), and water (to 6 oz.) may be prescribed.

Dyspepsia should be treated on general principles. A mixture of alkalies and hydrocyanic acid may be useful. Williamson recommends frequent 10 grain doses of bicarbonate of soda in a teaspoonful of milk. Sir W. Roberts gives a pill containing 2 or 3 grains of asofœtida to alleviate the craving for food and sense of sinking in the epigastrium.

For *flatulence and intestinal catarrh* an initial purge followed by a pill containing creosote, or thymol, and extract of belladonna may be prescribed. Salicylate of bismuth in 8 grain doses, with or without opium, twice a day is often useful in checking diarrhœa.

General itching of the skin may be relieved by sponging with tepid water, carbolic lotion (1 : 40), liq. carb. detergens ($\frac{1}{2}$ oz. to the pint), or a mixture containing acid hydrocyan. dil. (1 dram), glycerine (1 oz.), water (to 6 oz.). The best internal remedy is sodium salicylate in doses of 20 to 30 grains two or three times a

day. The condition is, however, most satisfactorily relieved by controlling the glycosuria.

Pruritis and eczema of the vulva, or prepuce, should be prevented by thoroughly drying the parts after each act of micturition, and reducing the sugar. Sodium salicylate internally will often relieve the pruritis. If eczema has actually developed it will be found that remedies which prevent the fermentation of the sugar and urine are the most serviceable. In my experience washing the parts with a mixture of yeast and water, a teaspoonful of fresh yeast in a pint of water, as recommended by Carnot, gives the best results. Washing with boracic lotion, or van Swieten's solution (1 part perchloride of mercury, 100 parts of alcohol, and 900 parts of water), and the subsequent application of boracic ointment, zinc ointment, or ung. conii, is often serviceable. A lotion containing sodium hyposulphite (1 in 40) is a favourite remedy with some, others recommend a 3 to 5 per cent. cocaine ointment, or a dusting powder containing 10 per cent. of orthoform. In obstinate cases the local analgesic action of X-rays has given relief. An ointment containing scarlet " R " has recently been recommended for diabetic ulcers and eczema.

Cystitis is best treated by washing out the bladder with a weak solution of sodium salicylate, and giving this drug, salol, urotropine, or helmitol internally.

Boils and carbuncles can only be satisfactorily treated when the sugar in the urine has been reduced, or caused to disappear, by the ordinary dietetic and medicinal measures. As a general tonic quinine, in 3 grain doses internally four times a day, may be helpful. Locally the ordinary antiseptic measures should be taken, but operation should be avoided if possible, especially if it involves the use of a general anæsthetic. Bier's hyperæmic treatment often gives very satisfactory results.

Gangrene.—The treatment of dry gangrene in diabetics does not differ from that usually adopted, except that the glycosuria must be controlled by diet, &c., and surgical interference be undertaken with care, especially if there is acetonæmia. Gangrene due to arterio-sclerosis most often occurs in the lower extremities, and the disease may affect but one or more toes. In these cases, if the line of demarcation forms early, if the adjacent parts are not inflamed, and if there is a good pulse in the posterior tibial artery behind the internal malleolus and in the dorsalis pedis, the removal of the dead portion alone may be sufficient. If, however, the line of demarcation forms slowly, if the foot is inflamed, and the pulse in the arteries mentioned is feebly felt, if at all, operations on the foot are harmful. In such cases the amputation should be above the

knee.   In the milder cases where amputation has been successfully
carried out the condition of the patient often improves to a sur-
prising degree, and cases have been reported by Koenig and others
in which the urine has become sugar-free.

As a rule, patients with moist gangrene are in an advanced stage,
and show well-marked evidence of acidosis, so that the risk of opera-
tion is great.   It frequently offers the only chance of prolonging life,
however, and should be undertaken if there is great pain in the
living margin of the tissue, or the glycosuria is increasing and coma
threatens, or there is marked fever and rapidly spreading cellulitis.
In the rarer type of case where acetonæmia is absent amputation
should be carried out as quickly as possible, and as wide of the
gangrenous area as can be managed.   Most scrupulous care must
be taken with the antiseptic details of the operation, and spinal is
preferable to general anæsthesia.   In either case the affected part
must be kept dry and aseptic until surgical interference becomes
necessary.   Herzfeld speaks highly of a dressing of dry sodium
perborate powder for diabetic gangrene and Dieulafoy has used hot-
air douches, at a temperature of 100° to 300° C., to prevent infection
and the subsequent dangers of septic intoxication and septicæmia,
with satisfactory results.

Nephritis.—When nephritis occurs as a complication of diabetes,
and is not due to the excretion of acetone bodies in the urine, the
nitrogenous food in the diet should be reduced, and replaced as
much as possible by milk and fats.   Although milk contains lactose,
many diabetics can take two or three pints a day without seriously
increasing the glycosuria.   Sugar-free milk may also be used, but
even this, in some instances, causes the excretion of sugar to rise.
It is consequently often a difficult matter to arrange the diet so
that the albuminuria will be decreased, without at the same time
augmenting the glycosuria.   The bowels should be kept well open
with saline purges, or senna, and a bottle or so of Vichy water should
be taken each day.   The use of opium and its derivatives is to be
avoided, but alkalies, and particularly sodium citrate, are often useful.

Œdema in the legs and other situations, not associated with
kidney lesions, is best treated by rest in bed.   Perchloride of iron
may also be given.   The œdema that sometimes results from the
oatmeal treatment usually diminishes rapidly when the nature of
the diet is altered.

Nocturnal cramp is most satisfactorily treated by controlling
the glycosuria, but relief may meanwhile be often obtained by the
administration of sodium bicarbonate in repeated doses of 10 or
15 grains, the last being taken at bedtime.

Neuritis and sciatica are treated on ordinary lines.   For the

gnawing pains in the legs of which some patients complain, Williamson advises antipyrin in 10 grain doses three times a day.

Sleeplessness may be combated by the exhibition of opium, sulphonal, and similar drugs, nervous excitement with potassium bromide.

Arterio-sclerosis and cardiac affections are treated by the usual remedies. For the former iodides are probably the most useful drugs, but attention must also be paid to the condition of the bowels. Digitalis should be used with great care in gouty glycosuria, in which the heart is hypertrophied and the arterial tension already too high. Its tendency to increase the tension may be counteracted by the simultaneous administration of nitro-erythrite or some similar drug.

Acidosis and Diabetic Coma.—Of all the complications of diabetes these are the commonest and most dangerous. The latter is, as we have seen, probably a result of the former, at any rate in most instances, so that the treatment of the one is involved in the treatment of the other. The use of carbohydrates has long been known to restrain acidosis, and in mild cases to cause its disappearance ; but such a line of treatment has the disadvantage that it is likely to ultimately do harm by making the glycosuria worse, unless it is carried out with great care. The amount of carbohydrate that can be utilised must be determined experimentally for each case in the manner already suggested, and the patient must be allowed this amount and no more. The assimilative powers can often be thus maintained for long periods, and even be improved, especially if regulated intervals of restricted diet are interposed. It is evident that sugar itself is not the body that controls acidosis, but that its action depends upon the formation of some derivative. Many observers have therefore devoted their attention to the search for this sugar product, or a substitute. A variety of substances, including gluconic acid, glutaric acid, alcohol, glycerine, and glycerine aldehyde, &c., have been found experimentally to reduce acidosis, but none have proved to be of practical clinical value, excepting perhaps alcohol. The toxic effect of alcohol has, however, to be reckoned with, and its use is therefore limited. As we have seen, the acids giving rise to acidosis originate from fats, and to a less extent from the fatty acid moiety of proteins. Since different fatty foods vary in the amount of acid they produce, those containing much of the lower fatty acids being especially harmful, the diet should be arranged to eliminate these as much as possible. With the exception of using fresh washed butter, or oleo-margarine, in the place of old butter, very little can be accomplished in this direction however. With regard to the nitrogenous

foods, it has been shown that they vary greatly in their influence on
acidosis, vegetable and egg proteins being less harmful than proteins
of animal origin.   Beside increasing the acids in the circulation,
proteins also give rise to substances that neutralise them—that is to
say, they are both ketogenic and anti-ketogenic.   The latter effect
depends partly upon the carbohydrate group that is split off and
is more easily utilised than ingested carbohydrate by all but the
most serious cases, and partly upon the amino-acid fractions which
exert a twofold action, some increasing the acidosis by yielding the
fatty acid of their molecule, while others have a restraining influence
from the ammonia that is derived from them.   Choice in the
selection of the nitrogenous part of the diet may therefore be used
to a certain extent to control acidosis, but the limits of selection
are too restricted to be more than occasionally of service in practice.
A high intake of protein may, according to Magnus-Levy, at times
increase acidosis, not from the formation of oxybutyric acid from
the protein, but because the high protein content of the diet makes
an extra tax on the oxidising powers of the organism and diverts
these from the combustion of acetone bodies.

The first indication, therefore, in the treatment of acidosis, and
its sequel diabetic coma, is to prevent or postpone their onset by
suitable qualitative and quantitative regulation of the diet, the
second is to neutralise the acids that form by the administration of
alkalies.   The latter, although a valuable method, is, after all, only
palliative, for alkalies do not limit the formation or favour the
combustion of acetone bodies.   In many cases, on the contrary, the
excretion of acetone bodies in the urine will increase if large quantities
of alkali are given.   This is not an unfavourable sign, but merely
indicates increased excretion.   I cannot too strongly insist on the
alsolute necessity for adapting the diet to the individual require-
ments of the case at the earliest possible stage if serious disturbances
of metabolism are to be avoided or controlled.

It must be remembered that acetonuria has not always the same
significance, and that cases presenting this sign may be con-
veniently divided into three classes, as v. Noorden suggests :—

(1) Cases of slight glycosuria, readily cured by the withdrawal
        of carbohydrates from the diet, but presenting shortly
        after this diet has been instituted signs of acetone in the
        urine.   In such cases the acetonuria is physiological and
        need cause no alarm, nor does it necessitate a withdrawal
        of the severe diet, as it will disappear in a few weeks.

(2) Diabetes with slight glycosuria that, in spite of a partially
        restricted diet, show signs of acetone, which, however,
        disappear on a more rigid diet.   The extra rigidity in

diet does not excite the same metabolic disturbance as in the first class of case, where hardly any restriction of diet had been observed.

(3) Cases where the glycosuria is marked, and where cutting off the carbohydrates does not suffice, and reduction of proteids is also necessary. In all such cases prolonged administration of alkalies is strongly urged as they help the elimination of the acetonuric acid bodies and diminish the acetonæmia. It is necessary to avoid too sudden restriction of diet, and it may often be advisable to allow a few days' liberty, and so gradually work down to a hydrocarbon-free diet. In the worst forms even the small amount of carbohydrate coming to the liver via the portal vein does not suffice to restrain the production of acetone, and in these cases—with a persistent acetonuria—it is quite indifferent whether we withhold the carbohydrates from the food or not.

The alkali most commonly used in the treatment of acidosis is bicarbonate of soda. The amount of this in excess of two drams required to make the urine alkaline may, as we have seen, be taken as a rough index of the intensity of the condition. As a rule it is not advisable to render the urine distinctly alkaline, but to keep it just short of that point, as otherwise symptoms of depression are likely to supervene. When, however, the premonitory symptoms of coma appear the administration of the drug should be pushed. Hucard gives 2 to 10 drams daily, and v. Noorden half an ounce of bicarbonate of soda each day, with 45 grams of calcium carbonate added to make up for the loss of calcium salts in the urine. The administration of calcium salts is probably also useful for other reasons. Large doses of bicarbonate of soda are liable to produce diarrhœa, and this is counteracted to a certain extent by calcium ; moreover, calcium salts are known to combine with toxic substances and assist in their elimination from the body. It has been proved experimentally by Silvestin that the administration of calcium lactate permits otherwise fatal doses of strychnine to be given to dogs and cats, also that calcium salts increase the resistance of animals to the effects of injections of blood serum from cases of uremia and eclampsia, so that calcium salts may be of use in diabetes from this point of view also. Some observers have strongly advocated sodium citrate in conjunction with, or in place of, sodium bicarbonate. It is pointed out that it is not neutralised by the gastric juice, and is converted into bicarbonate in the blood where the alkali is most needed. Its taste is not obnoxious, it does not cause disturbances in the stomach, the appetite does not suffer,

and even large doses, 750 grains a day, do not cause diarrhœa. It is claimed by Lichtwitz that the elimination of nitrogen in the urine is increased by the citrate treatment. In my own practice I generally prescribe a mixture [1] containing :—

Sodium bicarbonate	.	.	.	gr.	10–20,	up to 225 gr. daily.
Sodium citrate	.	.	.	,,	5–10	,, 75 ,,
Calcium carbonate	.	.	.	,,	4–5	,, 45 ,,
Magnesium carbonate	.	.	.	,,	4–5	,, 45 ,,
Water	.	.	.	oz.	1	,, 10 oz. ,,

One or two bottles of Vichy, or Neuenhar, water may also be taken each day.

The total amount of alkali given in the day should not exceed about an ounce, as larger quantities are likely to depress the heart. An increase in the body weight, with œdema and ascites, is often observed when large doses of sodium bicarbonate are being taken by diabetics. On stopping the drug, or reducing the dose, the weight falls, and the œdema disappears. It has been shown by Widal, Lemierre, and Cotoni that these changes are only indirectly dependent on the alkali, and are directly produced by a retention of chlorides, as is the œdema of cardiac and renal disease.

Beside prescribing large doses of alkalies, the premonitory symptoms of coma must be met by a thorough clearance of the bowels by means of a mild purge. Castor oil, compound jalep powder, or calomel, followed by a saline, are probably the most useful. Lavage of the stomach may be carried out, if practicable, to assist in the removal of toxines. The patient should be kept in bed, and all sources of bodily or mental fatigue be carefully avoided. If these measures do not suffice to control the symptoms, intravenous injection of an alkaline solution must be resorted to, but it is important that this should not be delayed too long, as the best effects undoubtedly result before the patient becomes actually comatose. Until recently a weak solution of bicarbonate of soda has been almost universally used, one to three pints of a 2·5 to 5 per cent. solution being injected into a vein. Blum has, however, pointed out that there is a limit of safety to this method, and that alkaline solutions of too great a strength may produce venous spasm and various evidences of central nervous poisoning, such as convulsions, &c. Blum believes that it is the lack of balance in the solution that is responsible for its toxurity, for various ill effects have been noticed after the injection of so innocuous a compound as sodium chloride. To injections designed simply to introduce the neutral chloride into the circulation, potassium and calcium

[1] This has been put up in compressed tablet form for me by Messrs. Allen and Hanbury under the name of " Compound Neutralising Tablets."

chlorides can be added to balance its effect, but with alkaline injections this is not possible, as insoluble calcium carbonate is formed. Blum thinks that a 6 per cent. solution of bicarbonate of soda is the least harmful to use, provided that this has been previously boiled for a quarter of an hour to sterilise the solution, and to render it less toxic by converting the bicarbonate into sesquicarbonate, which, according to Stadelmann, is better tolerated than the carbonate, and contains more sodium than the bicarbonate. Lichtwitz claims that sodium citrate is better adapted for intravenous injection than the bicarbonate, as it has only a weakly alkaline reaction, and this can be neutralised by adding a little citric acid. Some observers have been content to use only a sterile normal saline solution (0·6 per cent.), but even this, unless prepared with absolutely freshly made distilled water, is, according to Hort and Penfold, liable to cause fever and other toxic symptoms. In such a serious condition as advanced acidosis and threatening coma, some risks must be taken, and, on the whole, Blum's method of injecting two or three pints of a boiled 6 per cent. solution of bicarbonate of soda appears to be the least objectionable. The solution should be at body temperature, and be given slowly and steadily, one-half to three-quarters of an hour being taken to administer two or three pints, so that a probably feeble heart shall not be overcharged. As a rule, copious diuresis follows, and the pulse becomes stronger and less rapid. Sometimes a certain amount of œdema, attributed by Widal to retention of chlorides, follows the injection, but it is of no special import. If the patient is comatose he may return to consciousness, and his mind become surprisingly clear, but usually it is only a temporary rally, and after a few hours a relapse takes place and death speedily follows. Very rarely the recovery is permanent. Oliver, for example, has recorded a case in which the patient left the hospital four weeks after an attack of diabetic coma treated with intravenous injections of sodium bicarbonate, and Labbé has reported one in which combined intravenous and oral administration brought about a cure. The patient, who had sunk into unconsciousness, received an injection of 15 grams of bicarbonate of soda, and was sufficiently restored to take 60 grams by the mouth. For five consecutive days she was given injections, and she was then so much better that the injections were stopped, and the treatment was continued only by the mouth. Two days later the coma returned ; she was then given an intravenous injection of 30 grams, repeated the next day, and after a month's alkaline treatment by the mouth she was completely cured. Bleeding from the opened vein to the extent of about a pint, before the injection is made, may assist in overcoming the toxæmia.

Sicard has recently advocated the use of intravenous injections of sterilised, concentrated (8 to 9 per cent.) solutions of sodium bicarbonate in the treatment of other symptoms than acidosis, when these resist the ordinary dietetic and medicinal measures. In three cases, one of obstinate pruritis, another of sciatic pains, and a third of optic neuritis, he obtained marked remission. He injects from 100 to 250 c.c., representing about 20 grams of sodium bicarbonate, into a vein in the arm, but points out that it is absolutely necessary that the injection should be made directly into the interior of the vessel as the solution is caustic, and if introduced into the cellular tissue would produce an inflammatory reaction. The injection is extended over about five or ten minutes. He states it may be repeated several times, if necessary, at intervals of a few days without the least danger.

To avoid the discomfort and disturbance consequent on the ingestion of large quantities of sodium bicarbonate by the mouth some physicians employ rectal injections ($\frac{1}{2}$ oz. in $\frac{1}{2}$ a pint) with, or without, the addition of dextrose (1 dram) in cases of impending coma every four hours, but as diarrhœa and tenesmus are very likely to result it is not a method to be recommended.

When coma is imminent, or the patient is actually unconscious, cardiac stimulants such as alcohol, ether, strychnine, caffeine, ammonia, and digitalis should be given by the mouth, or be administered subcutaneously. Some authors have advised oxygen inhalations with a view to combating the air-hunger, but, according to Pembrey, this procedure is unnecessary and useless, as the alveolar air contains plenty of oxygen (17 to 18 per cent.), and the deep breathing is merely a response to the stimulating action of the acid substances present in the blood.

Treatment of Infantile Diabetes.—Glycosuria is a rare condition in young children, but it is probable that it would be found more commonly than is generally thought if it were sought for systematically. It is particularly important that it should be recognised and come under treatment as early as possible, since in infants it is an acute disease that may terminate fatally in a few days, and even in older children it may run its course in a few months. As a rule, the onset and development of diabetes are not quite the same in children as in adults. In addition to polyuria there is generally incontinence ; thirst is a very prominent symptom in nearly all cases ; the urine is often alkaline, and contains a high proportion of sugar (30 to 80 grams per litre), and a large quantity of urea (20 to 25 grams in the twenty-four hours). The onset of the

condition is usually insidious, and is often confused with digestive or dentition disturbances, attention being first directed to the true state of affairs by the thirst, polyuria, and loss of weight.

The treatment consists in reducing the ingestion of foods containing dextrose, and improving metabolism generally as much as possible. With breast-fed children nursing is continued, but a small teaspoonful of Vichy water is given after each feed. With artificially fed children the milk is sweetened with mannite, glycerine, or saccharine, and is diluted with Vichy water. The patient may also be fed with a milk specially prepared as follows : The casein and fat of one and a half litres of ordinary milk are precipitated by adding rennet, the sugar is removed by washing the coagulated mass, and the latter is then pressed through a fine sieve into a mixture of 200 grams of whey and 1300 grams of water. The mixture obtained in this way contains only 8 grams of sugar, but much fat and albumen. It may be made more palatable by adding saccharine. When a breast-fed child is weaned it is nourished on milk, or cream, diluted with water, or Vichy water, and sweetened with glycerine or saccharine. Eggs, minced meat, and green vegetables should be gradually added to the diet. Starchy foods must be avoided as much as possible, and as soon as it is old enough the child should be given prepared vegetable proteins (e.g. Roborat) and eggs. As a precaution against acidosis the nitrogenous diet should not be too rigid, or be too quickly introduced. Fatigue of all kinds should be guarded against. An older child should have the first meal in bed at a fixed time. He should remain in bed till mid-morning, and then be allowed to be up and out until the hour for the noon meal, after which he should be well wrapped and made to rest quietly in the open air for at least two hours. He may then be given mild exercise, walking or driving, if in good condition, or massage if passive exercise is better borne. Supper should be not later than 6 P.M. and bed at 7. The sleeping room should be ventilated as for a tuberculosis case. As much care must be taken to guard against waste of nervous energy, and to provide fresh air at all times, as is used in regulating the food. The patient should be put on a standard diet, suited to his age and condition, and as free as possible from carbohydrate. A supplementary diet consisting principally of the carbohydrates found to be best borne should be held in reserve, from which is added more or less according to the varying carbohydrate tolerance shown.

The urine must be examined as often, and as thoroughly, as possible, and the patient be weighed every three or four days.

Drugs are to be used with care, quinine, iron, cod-liver oil, alkalies, arsenic, and bromides being the most useful. Where there is evidence of specific disease mercury should be prescribed. For infants, waters containing alkalies, chlorides, and arsenic are the most valuable. Alkaline waters also benefit older children, but when a stimulating effect is desired waters containing iron and chlorides should be employed. Constipation must be carefully guarded against, and diarrhœa must be promptly checked. When coma is threatened the symptoms are met in the usual way with purgatives, stimulants, and alkalies. Very satisfactory results have been obtained in diabetic children with v. Noorden's oatmeal cure, especially when it has been instituted at an early stage. Abt and Strouse have reported that in two cases of traumatic diabetes in children that they treated by this method, carbohydrate tolerance was strikingly raised and the acidosis was considerably reduced.

Prophylactic Treatment.—As our knowledge of the pathology and etiology of persistent glycosuria grows, it becomes increasingly clear that, not only can no hard and fast line be drawn between simple glycosuria and glycosuria with secondary disturbances of metabolism, but that in many instances sugar is intermittently present in the urine before permanent glycosuria is established, and also that there is probably what may be called a " pre-glycosuric stage " in which carbohydrate metabolism, although disturbed, is not so far interfered with that sugar appears in the urine when an average amount only of carbohydrate is consumed. It is the experience of all who have had to deal with many cases of diabetes, that the earlier treatment is commenced the more satisfactory and permanent are the results, and we may, therefore, conclude that if patients could be treated in the pre-glycosuric stage the onset of glycosuria might be much delayed, or even be prevented. Our knowledge of the etiology of many of the pathological changes that give rise to glycosuria is so meagre that, at present, we can accomplish little in this direction, except in a general way. It is known, for instance, that sugar may appear in the urine of obese and gouty individuals, so that if patients suffering in these ways were watched and treated with the possibility of glycosuria occurring as a complication in mind, it might often be prevented. Again, the tendency to defective carbohydrate metabolism appears to be in-herited in some families, and it is likely that much might be done to hinder or prevent its development if the metabolism of such in-dividuals were periodically investigated, especially in early life, and they were warned against conditions that are known to play a

part in the production of persistent glycosuria. The absorption of indican, and other products of protein putrefaction, has been stated to affect the supra-renals, and so may bring about a disturbance of the balance between the ductless glands that normally controls carbohydrate metabolism. It would, therefore, seem advisable that intestinal catarrhs, and similar conditions that are associated with abnormal putrefactive changes in the intestinal contents, should be recognised and controlled as quickly as possible.

As a result of the work of numerous observers we are now on surer ground with regard to diseases of the pancreas than was the case some years ago. In a paper that I published in 1908 I pointed out that :—" So long as the etiology of many lesions of the pancreas was obscure, and their diagnosis a matter of extreme difficulty, little could be done to deal with them before they had advanced to such a stage that the functions of the gland were hopelessly impaired, but now that we can readily detect the probable presence of degenerative changes by means of the " pancreatic " reaction in the urine, and a consideration of the clinical history and symptoms, together with the results of a careful examination of the urine and fæces, will usually serve to throw light on the cause of the condition, there can be no reasonable excuse for allowing the disease to progress undiagnosed and untreated until diabetes supervenes." My experience since these words were written has not given me any reason to alter the opinion I then expressed ; in fact, I feel even more confidence in stating that if pancreatic diseases, and particularly those of an inflammatory type, were more generally recognised in the early stages, and were thoroughly treated, the number of cases of diabetes with which we have to deal would be considerably reduced. As a result of the opportunities that I have had during the last ten or twelve years of observing a large number of individuals suffering from pancreatic disease and diabetes, I have been led to divide cases of pancreatic glycosuria into three classes according to the probable source of the morbid influence affecting the pancreas :—

(1) Those in which the pancreatic mischief is probably secondary to some morbid influence reaching the gland by way of the ducts. (2) Those probably secondary to blood diseases or circulatory disturbances, including arterio-sclerosis, interacinar pancreatitis, syphilis, &c. (3) Those in which the diabetes was induced by destruction of the pancreas by malignant disease, either primary, or a secondary invasion from neighbouring organs.

My object in doing so has been to make clear their etiology, and so open the way for earlier and more radical treatment. In

the first class there is usually a long antecedent history of
" dyspepsia," or gastro-intestinal trouble, probably indicating a
duodenal catarrh, or of attacks of jaundice of the " catarrhal
type," or of gall-stones. The progress of the disease is slow, and
it is not until the pancreatic lesion has reached an advanced stage
that glycosuria occurs. I have met with cases in which this was
shown by several years intervening between the discovery of the
pancreatitis and the appearance of sugar in the urine. One patient
who had been operated on for gall-stones did not develop glycosuria
until eight and a half years after the operation. In another the
symptoms of diabetes came on four years after an exploratory
operation for what was believed to be cancer of the pancreas. At
the time these operations were performed chronic pancreatitis, and
its possible consequences, were not fully recognised, but had means
been taken then to stay the progress of the disease it is likely that
a cure might have been effected. The striking increase in the
death-rate from diabetes shown in recent years by the Registrar-
General's returns, and also in the mortality tables from Paris and
New York, is probably not unconnected with the greater prevalence
of digestive disturbances :—

Death-rate from Diabetes per 100,000 *Population*

				London	Paris	New York
1880 .	.	.	.	4·3	5·0	5·71
1890 .	.	.	.	6·6	13·0	8·06
1900 .	.	.	.	7·7	15·8	11·34

A detailed study of these returns shows that the increase is
chiefly due to the larger number of cases occurring in older people
at ages from fifty-five to seventy-five, or at least not causing death
before the later periods of life ; that is to say, the glycosuria makes
its appearance at a time when we might expect the secondary effects
of intestinal disturbances and intestinal toxæmias on metabolism
to become apparent. I would, therefore, urge that when symptoms
of dyspepsia are combined with the presence of the " pancreatic
reaction " in the urine, prompt steps should be taken to deal with
the pancreatitis that is probably present, lest, in the course of time,
worse should befall. In addition to the routine medical treatment
of such cases I make use of hexamethyleneamine (urotropine),
salicylate of soda, and intestinal antiseptics, such as sulphocarbolate
of soda, or izal, with a view to controlling the infection of the

pancreatic ducts. Physiological rest is also given to the pancreas by a carefully selected diet, and by placing the meals at the longest possible intervals from each other. If, after a fair trial, these methods are found to fail, and the " pancreatic reaction " persists as markedly as ever, recourse must be had to surgery. The pancreatic ducts must be drained by cholecyst-enterostomy, and, if necessary, a gastro-enterostomy will give rest to the inflamed duodenum. Diabetes is not a common result of obstruction of the common duct by gall-stones, but it does follow in a certain proportion of cases, and it is impossible to foretell what will result in any particular instance. I have already mentioned one case in which glycosuria developed eight and a half years after an operation in which, although the gall-stones were removed, no provision was made for dealing with the pancreatitis that was present ; and I have met with similar cases in which sugar was found three or four years after an operation for gall-stones in the common duct. Chronic pancreatitis is found to be associated with about 70 per cent. of cases of common duct cholelithiasis, and as medical treatment has little or no effect on the disease, it is important that all gall-stone cases should be operated on at the earliest possible moment, particularly if there is a well-marked " pancreatic reaction " in the urine. A small amount of sugar is no bar to operative interference ; in fact, it may result in the disappearance of the glycosuria. Both pancreatic calculi and cysts are probably the result of catarrhal pancreatitis, due to an infection of the ducts from the duodenum, and although no radical benefit can be expected to follow the treatment of the diabetes occasionally found to complicate these conditions, much may be done to prevent their onset and to avoid a consequent glycosuria by timely recognition of the chronic pancreatitis that precedes and accompanies them.

The treatment of cases belonging to the second and third classes calls for no special remark. The etiology of arterio-sclerosis and interacinar pancreatitis is alike obscure, and, until we have further information with regard to them, empirical methods of treatment are all that can be adopted. Syphilitic patients with diabetes are said to have been cured by anti-syphilitic treatment, but, as a rule, the result is not satisfactory. The arterio-sclerotic type of diabetes is the most hopeful. It is the form most commonly met with in elderly people, and is probably due to circulatory disturbances in the pancreas. It is not infrequently associated with granular kidney and traces of albumen in the urine. These patients usually respond well to dietetic and general hygienic treatment, carefully applied according to the requirements of the individual case. All

the cases I have had the opportunity of observing have much improved under treatment.

Diabetes due to destruction of the pancreas by malignant disease, either primary or secondary, is beyond all forms of satisfactory treatment.

Surgical Treatment.—It was for long one of the accepted axioms of surgery that operative treatment of patients whose urine contained sugar should be avoided unless absolutely necessary, but with the introduction of antiseptics one of the most serious dangers attending surgical interference in such cases was done away with. Owing to their disturbed metabolism, imperfect nutrition, reduced resistance, impaired reparative processes, and depressed nervous system the most careful consideration is still called for before operation is decided on, but if proper precautions are taken, and the patient is carefully prepared beforehand, a large proportion of diabetics can be operated on as safely and as satisfactorily as other individuals. The three chief dangers that have to be contended against are (1) sepsis, (2) coma, and (3) failure of the wound to heal. By a rigid application of the principles of aseptic or, probably better in these cases, of antiseptic surgery the first complication can no doubt be avoided. The second is more difficult to contend against. If operation becomes imperative in a patient with acetonæmia a very considerable risk is always run, especially if the operation is prolonged and a general anæsthetic employed. It would seem advisable that, whenever possible, spinal anæsthesia should be used. When time can be given to the preparation of the patient the risk of coma can be considerably reduced by regulating the diet, securing thorough evacuation of the bowels, and administering calcium salts and alkalies until the reaction of the urine is neutral or nearly so. Should symptoms of acidosis develop subsequent to the operation they should be met by the administration of alkalies by the mouth, or by rectal or intravenous injections. Von Noorden's oatmeal diet has been recommended after operation as a preventive of acidosis. In my experience failure of the wound to heal is often the most serious difficulty. I know of several cases in which the wound showed no sign of union a week or more after operation.

In addition to what may be termed operations of necessity, such as those for diabetic gangrene, &c., operations of choice have to be considered. In these there is either a tumour that needs removing or some complication, such as duodenal ulcer, empyema, or gall-stones, &c., the surgical treatment of which will probably

give a better chance of recovery. Each case must be considered from every point of view before it is decided that operation is, or is not, advisable, but the dominating factor is the condition of the urine, and particularly the degree of acidosis and the way it responds to treatment. In my opinion the amount of sugar is not of itself a question of supreme importance, for many patients with well-marked glycosuria can be safely operated on, provided that secondary disturbances of metabolism are absent or are only slight. Moreover, the removal of a tumour which is possibly interfering with the blood supply of the pancreas, or of gall-stones that are keeping up a catarrhal inflammation of the gland, may cause the sugar to disappear or be materially reduced in amount. Evelt and Henkel, for example, have reported cases of ovarian tumour in which the removal of the growth was followed by a complete disappearance of the sugar that had been previously present in the urine. Henkel had the same experience with a case of uterine myoma. Carey Evans has described a case in which the glycosuria disappeared after gastro-enterostomy for severe indigestion lasting for eighteen months. Mayo Robson and Mansell Moullin have published records of cases in which glycosuria has been apparently cured by the removal of gall-stones from the common bile duct. It must not, however, be too readily assumed that because no sugar is found in the urine shortly after operation that a cure has been effected, for it may be that it will return when the patient resumes his ordinary diet and mode of life, or that the improvement is only a temporary one, so that, unless the patient is treated as a potential diabetic, the damage done to the pancreas will progress, and eventually give rise to incurable glycosuria. I have met with two cases reported as cured by operation who subsequently relapsed, and one of them to my knowledge died of diabetic coma.

The need for a more general early recognition and treatment by surgical means of interstitial and catarrhal pancreatitis was emphasised by Mayo Robson in a paper published in 1910. He pointed out that although we cannot hope to cure fibrosis of the pancreas by surgical interference, it is possible to remove some of the exciting causes of the antecedent inflammation and so rescue a sufficient amount of gland substance in a functionally active condition to cure an existing glycosuria, or prevent the subsequent onset of diabetes. The view is an attractive one, and well worth the serious consideration of both physicians and surgeons.

Prognosis.—Persistent glycosuria undoubtedly shortens life, but different cases vary so much in their gravity that the prognosis

in any particular instance cannot be based upon a statistical estimate of the average duration of the disease. Each case must be separately considered. The principal points to be taken into account in giving a prognosis are :—

(1) The age and sex of the patient, (2) the state of nutrition, (3) the cause of the glycosuria, (4) the total amount of sugar excreted daily in the urine, (5) the presence and degree of secondary disturbances of metabolism, (6) the response to treatment, (7) the social condition of the patient, (8) the nature and extent of any complications.

(1) *Age* influences the prognosis in diabetes to a marked degree, the outlook being generally worse the earlier in life the glycosuria makes its appearance. If it begins in the second half of life it is often a comparatively harmless disorder, but in children it is usually a very grave condition, the younger the child the shorter being the duration of the illness as a rule. It must not be concluded, however, that diabetes in a child is of necessity always rapidly fatal, for Hédon, Abt and Strouse, Crofton, and others have reported cases in which glycosuria in children as young as three months has been kept in check by treatment for a period which, in one instance, extended to twenty-five years. The varying course run at different ages probably depends on the fact that in early life provision has to be made, not only for the maintenance of the tissues, but also for their growth, hence the energy requirement is relatively high, and should the organs of metabolism be congenitally weak, or diseased, they quickly give way under the double strain ; in adult life sufficient energy to maintain life only has to be provided, so that the loss of sugar can be better borne ; in old people the activities of the body are normally lower than in early life, hence less energy is required and its loss in the shape of sugar in the urine can be still better withstood.

Sex.—If a number of cases of diabetes are observed it will usually be found that the mortality is higher among the females than among the males. Thus Laache in his experience of 122 cases states that only seventeen out of seventy-seven male patients died during the time, that forty out of forty-five cases in women terminated fatally. The greater mortality among females is probably to be explained by the fact that diabetes is more common among young women than among young men, and hence runs a more malignant course.

(2) *The general nutrition* of the patient is an important consideration in forming a prognosis. As a rule thin, ill-nourished individuals respond badly to treatment, and are very liable to develop acidosis

and succumb to diabetic coma, while in stout persons, and those who are not much wasted, the course of the disease is usually slower, and is more readily controlled by diet, &c. The former, too, are more liable to develop fatal complications such as tuberculosis.

(3) *The cause* of the glycosuria cannot be determined with any degree of certainty in many cases, but in others there is a probable explanation, and in a few it can be definitely ascertained. The prognosis in traumatic glycosuria is very frequently good, for the symptoms often disappear spontaneously, or are transformed into those of diabetic insipidus. Even in children this form of glycosuria sometimes responds well to treatment. Pancreatic diabetes is generally regarded as a very grave condition. Some forms, and more particularly those secondary to intestinal troubles, interacinar pancreatitis, and of course malignant disease of the pancreas undoubtedly run a comparatively rapid course, but the variety due to interlobular fibrosis that is associated with gall-stones, typhoidal, and other forms of cholangitis, pancreatic cysts and calculi usually progresses slowly, particularly if the primary source of the trouble can be removed. It is in the latter type that analysis of the fæces shows imperfect digestion of fats, a positive pancreatic insufficiency test, &c., and from the results of such an analysis it is possible to gauge the extent of the cirrhotic changes in the pancreas, and so form an opinion as to the probable duration of the disease. Gouty diabetes, and glycosuria associated with arteriosclerosis, are usually not grave conditions, provided that the patient is in a good state of nutrition for his age, and will submit to treatment. Glycosuria in obese individuals also runs a benign course as a rule, if properly treated. Inherited infantile diabetes is a very grave condition, terminating fatally in a few weeks to eight months in thirty-seven out of forty-three cases collected by Lion and Moreau. Once installed in a family the disease menaces all the children, but the cases on record in which one or more have escaped show that it does not necessarily affect every individual of the family. The prognosis for persons belonging to families in which an inherited tendency to glycosuria does not show itself until adult life is much more hopeful, especially if it is not apparent until after middle age is reached and is associated with gout, asthma, &c., in the individuals affected, or in other members of the family.

(4) *The Daily Excretion of Sugar.*—The gravity of a case of diabetes is very commonly estimated by the percentage of sugar contained in the urine, but this is a most fallacious guide, even when the figure is determined from an analysis of the mixed daily excretion. A

better, but still misleading, way is to calculate the total output
for the twenty-four hours. An accurate estimate of the true state
of carbohydrate metabolism can only be arrived at by determin-
ing the relation existing between the total output of sugar in
the urine and the total intake in the food, including in the latter
the possible maximum of sugar that can be derived from the protein
of the diet,&c., in the manner already explained. It is thus recognised
that the amount of sugar in the urine depends both on the quantity
of carbohydrate ingested, and upon the height of protein meta-
bolism. In severe cases where the power of utilising sugar, in-
cluding even that derived from proteins, is entirely, or almost
entirely, lost the figures representing the intake and the output
will be nearly the same, but in less serious cases the intake will
exceed the output by an amount varying with the extent to which
the power of metabolising carbohydrates is retained. The re-
lationship is most conveniently expressed by Falta's, or Lusk's,
coefficient of excretion.

Another method of estimating the gravity of diabetes has been
suggested by Mendel and Lusk as the result of their observations
on the total nitrogen and sugar contents of the urines of animals
with severe experimental glycosuria. The patient is given a
meat-fat diet (rich cream, meat, butter, and eggs) and the twenty-
four hours urine of the second day collected in such a way that the
final specimen shall be taken at an early morning hour (before
breakfast). The discovery of 3·65 grams of dextrose for each gram
of nitrogen in the urine indicates complete intolerance for carbo-
hydrates, and probably a quickly fatal termination. The authors
have consequently called this (D : N : 3·65 : 1) " the fatal ratio."
A lower ratio of dextrose to nitrogen on this diet shows that some
protein sugar is being utilised, and the prognosis is more favourable.

(5) *Secondary Disturbances of Metabolism.*—Quite as significant
as the assimilative capacity for sugar, is the quantity of organic
acid in the urine. A well-marked reaction for aceto-acetic acid
has long been accepted as an unfavourable sign, especially if it does
not disappear when the patient is carefully dieted. But the presence
of acetone alone does not necessarily mean there is a serious degree
of acidosis. A more reliable opinion as to the probable outcome
of the case can be arrived at by looking for and estimating the
oxybutyric acid. Whenever a patient regularly passes more than
5 grams a day the prognosis is bad, for he is liable at any time to
become comatose. A decline or disappearance of the acid on a
suitable diet is a favourable sign, but its gradual increase, in spite
of dietetic regulations, is a bad omen. A patient may live for

many months, however, in spite of his urine containing an amount of acid equivalent to 15 or 20 grams of oxybutyric acid a day. An indication of the degree of acidosis and associated secondary disturbances of metabolism is more readily obtained by estimating the amount of ammonia nitrogen in the twenty-four hours urine ; if this is found to be 3 grams, or over, the prognosis is unfavourable, and coma is probably imminent.

(6) *Response to Treatment.*—Many cases of chronic glycosuria which at first sight appear hopeless, will, under suitable dietetic, medicinal, and hygienic treatment, improve in a most remarkable manner. It is, therefore, never safe to give a definite prognosis until the patient has been under observation for some time. Many physicians divide their cases into " mild " and " severe " according to the way they respond to a test-diet, such as that of v. Noorden. This consists of meat, eggs, bacon, butter, green vegetables, cheese, lettuce, salad, coffee, and wine. At breakfast and lunch 50 grams of white bread are allowed. Should the urine be sugar-free on such a diet the diabetes is of a " mild " type. If sugar is present the quantity of bread is gradually reduced, and if the glycosuria still persists after all the bread has been removed from the diet the case is regarded as one of " severe " diabetes.

(7) *The social position* of the patient is chiefly of importance because the wealthier classes are able to devote time and money to their cure which it is impossible for persons lower in the social scale to expend ; moreover, people belonging to the higher grades of society are, as a rule, more intelligent, and it is consequently possible to impress them with the necessity of strictly carrying out the line of treatment decided on. Their surroundings, too, are usually more conducive to healthy metabolism and the avoidance of infections. Hence, other things being equal, a person of good social position has a better chance of life than one belonging to the lower classes of society.

(8) *Complications.* — Certain complications are particularly dangerous to life in diabetics. Pneumonia, for example, is a much more fatal disease than it is in healthy individuals. Pulmonary tuberculosis progresses very rapidly and is hardly ever materially affected by treatment. Moist gangrene is, as we have seen, a most serious complication, and makes the prognosis much more grave, but is not necessarily hopeless. In women who are diabetic and become pregnant, labour is the great danger to be feared, especially if it is prolonged.

BIBLIOGRAPHY

Abelmann, *Dissertation*, 1890.
Abt and Strouse, *Amer. Journ. Med. Sci.*, 1911.
Allan, *Lancet*, 1904.
Bainbridge, *Biochem. Journ*, 1908.
Bainbridge and Beddard, *Biochem. Journ.*, 1906.
Beardsley, *Therap. Gaz.*, 1911.
Blum, *Semaine Méd.*, 1911.
Board U.S. Dept. Agriculture, *Rep.* 94, Washington, 1911.
Bruce, *Practitioner*, 1887–8.
Brück, *Mediz Klinik.*, iv.
Caessaet, *Semaine Méd.*, 1875.
Cammidge, *Surg. Gynec. and Obst.*, 1908.
Cantini, *Spec. Path. u. Therap. d. Stoffwech.*, 1880.
Carnot, *Progrés Médicale*, 1910.
Cavazzani, *Arch. d. clin. méd.*, 1893.
Charles, *Bristol Med. Chi. Journ.*, 1906.
Cowles, *Boston Med. and Surg. Journ.*, 1911.
Crofton, *Amer. Journ. Med. Sci.*, 1902 ; *Philadelph. Med. Journ.*, 1902 ; *Amer. Med.*, 1902 ; *Lancet*, 1909, 1911.
Crow, *Johns Hopk. Hosp. Bull.*, 1908.
Dickinson, *Dis. of Kidneys*, 1875.
Dieulafoy, *Acad. d. Méd. d. Paris*, 1910.
Dujardin-Beaumetz, *Soc. d. Thérap.*, 1888.
Ebstein, *Die Zuckerkrank*, 1887.
Evans, *Lancet*, 1907.
Evelt, *Monat. f. Geb. u. Gyn.*, 1907.
Falta, *Arch. int. Med.*, 1909.
Feinberg, *Jahresb. u. d. Leitsung.*, 1889.
Forchheimer, *Amer. Journ. Med. Sci.*, 1911.
Forschbach, *Deut. med. Woch.*, 1909.
Foster, *Journ. Biolog. Chem.*, 1907.
Le Gendre, *Journ. d. Mèd.*, 1911.
Gönner, *Correspbl. f. Schweiz. Aertze*, 1887.
Grübe, *Münch. med. Woch.*, 1895.
Guelpa, *Auto-intox. et désintox.*, 1910.
Hédon, *Physiol. Normale et Path. d. Panc.*, *Compt. rend. d. Soc. d. Biol.*, 1911.
Henkel, *Deut. med. Woch.*, 1909.
Herter, *Lectures on Chem. Path.*, 1902.
Herzfeld, *Journ. Amer. Med. Assot.*, 1911.
Hirschfeld, *Berl. klin. Woch.*, 1910.
Hort and Penfold, *Brit. Med. Journ.*, 1911.
Hucard, *Rev. gén. d. Chem. et d. Therap.*, 1893.
Jennings, *Lancet*, 1911.
Koenig, *Berl. klin. Woch.*, 1896.
Laache, *Mediz. Klinik.*, 1910.

Labbé, *Arch. gén. d. méd.*, 1911.

Landergren, *Nord. med. ark.*, 1910.

Lépine, *Diabète Sucré*, 1909.

Leschke, *Münch. med. Woch.*, 1911.

Lichtwitz, *Therap. Monatsch.*, 1911.

Lion and Moreau, *Arch. d. Méd. d. Enfants*, xii.

Locke, *Food Values*, 1911.

Löfer, *Berl. klin. Woch.*, 1911.

Lusk, *Journ. Amer. Med. Assoc.*, 1910.

Levy, *Johns Hopk. Hosp. Bull.*, 1911.

Mendel and Lusk, *Deut. Arch. f. klin. Med.*, 1904.

Minkowski, *Arch. f. exp. Path. u. Pharm.*, 1908.

Moore, Eden, and Abram, *Biochem. Journ.*, 1906.

Mossé, *Rev. d. méd.*, 1902.

Mosenthal, *Journ. Amer. Med. Assoc.*, 1912.

Moullin, *Lancet*, 1907.

Neumann, *Zeit. f. klin. Med.*, 1910.

Von Noorden, *Centralb. f. inn. Med.*, 1895 ; *Handb. d. Path. d. Stoffwech.*, 1907 ; *Berl. klin. Woch.*, 1903 ; *Rif. Med.*, 1911.

Oliver, *Lancet*, 1898.

Pembrey, *Brit. Med. Journ.*, 1910.

Philip, *Brit. Med. Journ.*, 1910.

Ralfe, *Lancet*, 1892.

Rédon, *Thésis*, Paris, 1877.

Renne and Fraser, *Biochem. Journ.*, 1907.

Richardiere, *Union Méd.*, 1895.

Robson, *Brit. Med. Journ.*, 1910.

Rudisch, *Journ. Amer. Med. Assoc.*, 1909.

Schmitz, *Zuckerkrank*, 1892.

Sewall, *Amer. Journ. Med. Sci.*, 1911.

Sicard, *Soc. méd. d. hôp. d. Paris*, 1911 ; *Journ. Amer. Med. Assoc.*, 1911.

Silverstin, *Gaz. d. Osp. e. d. Clin.*, 1911.

Spooner and Pratt, *Journ. Amer. Med. Assoc.*, 1910.

Thompson and Wallace, *Brit. Med. Journ.*, 1911.

Wallace, *Journ. Amer. Med. Assoc.*, 1910.

Warden, *Lancet*, 1911.

Weintraud, *Therap. Monats.*, 1908.

West, *Brit. Med. Journ.*, 1895.

Widal, Lemierre, and Cotoni, *Semaine Méd.*, 1911.

Williams, *Brit. Med. Journ.*, 1894.

Williamson, *Diabetes Mellitus*, 1898.

Zuelzer, *Deut. med. Woch.*, xxxiv.

CHAPTER X

OTHER CARBOHYDRATES MET WITH IN DIABETIC URINES—
LEVULOSURIA, MALTOSURIA, ETC. ETC.

Levulosuria.—Owing to reliance being placed on methods of analysis which are now regarded as open to objection, many of the earlier recorded cases of levulosuria are of doubtful character, but the tests employed by recent investigators have been such that there can be no doubt that levulose does appear in the urine spontaneously, both alone and along with dextrose.

According to modern research three forms of levulosuria exist—(1) alimentary, (2) pure spontaneous, (3) mixed, in which there is more or less dextrose along with the levulose.

1. *Alimentary levulosuria* has been already considered (p. 164), so that it will not be necessary to deal with it in detail. It will be remembered that, according to v. Noorden, the assimilation limit for levulose is about 200 grams. In some individuals, however, it is lower than this, 100 grams, or less, causing levulose to appear in the urine. Alimentary levulosuria is rare in health, but examples have been reported in apparently healthy persons by Moritz, Haycraft, and Strauss. Of much greater interest than these physiological curiosities is the proved association of alimentary levulosuria with functional disturbances of the liver, and, although levulosuria is not pathognomonic of serious hepatic mischief, its presence in any particular case is of considerable diagnostic value. Levulose is a sugar that is frequently well assimilated by diabetics, but alimentary levulosuria is not uncommon in diabetes, both in chronic cases and also in the recent severe type. In both the failure to make use of levulose is probably dependent upon functional derangement of the liver, and in some is associated with actual structural change. Cases of this description have been recorded by a number of observers, including among the more recent Borchardat, Graul, Schwarz, and Schleisinger.

2. *Pure spontaneous levulosuria* is a rare condition. Cases apparently belonging to this category have been described by Ventzke, Cotton, Personne and Henniger, Seegen, Külz, Carles, Marie and Robinson, Rosin-Laband, Schleisinger, Lépine and

Boulud, Schwarz, Neubauer and Moraczewski, but the evidence in support of some of them at least is doubtful.

The quantity of levulose excreted has always been small, rarely exceeding 1 or 2 per cent., with a total daily excretion of 20 or 30 grams.   Seegen, for example, found up to 2 per cent., Lépine up to 2·4 per cent., Neubauer 1·8 per cent., Schwarz 0·3 per cent., but as they do not state the total amount of urine passed the daily excretion cannot be calculated ; in Rosin's case, however, 22 grams were excreted in the twenty-four hours, and in Schleisinger's 3 to 4 grams.

3. *Mixed levulosuria and dextrosuria* is a much more common condition than pure levulosuria ; but there is a very wide divergence of opinion among different observers as to the frequency with which levulose is met with in the urine of patients suffering from persistent glycosuria.   According to Rosin, dextrose and levulose are found together " very often," Umber considers that they are " not seldom " associated, Schwarz found levulose in the urines of six out of nineteen cases of diabetes, Schleisinger in three out of eighteen ; but in all of them there was a large amount of sugar in the urine, owing to their not having been properly treated.   Umber states that slight spontaneous levulosuria is so common in cases recently admitted to hospital that it is probably physiological, and, as it soon disappears on a careful diet, it is probably derived from the food.

In many instances statements as to the presence of levulose in the urine of cases of persistent glycosuria have been based entirely upon the difference between the amount of sugar shown by titration and polarisation and the presence of a positive Seliwanoff reaction, but such evidence is not of itself conclusive.   In the first place, the accuracy of the ordinary titration methods for sugar in the urine is not by any means as great as is generally assumed, and a difference of a $\frac{1}{2}$ to 1, or even 2, per cent. between the figures so obtained and those given by the polariscope may easily be due to experimental errors and the presence of other reducing substances. Again, it must be remembered that albumen, β-oxybutyric acid, glucuronic acid compounds, and cystin are levo-rotatory and that their presence must be excluded, or allowed for, when the polariscope is being used for the detection and estimation of sugar. Seliwanoff's test, unless very carefully carried out, is very liable to give misleading results, and is, moreover, not strictly specific for levulose.   Realising these sources of possible error, trustworthy investigators now usually confirm their results by separating the levulose as the methylphenylosazone, or as a calcium, or other

comparatively insoluble, compound. When examining a urine for levulose it must be borne in mind that, according to Külz, the levo-rotatory sugar met with in diabetic urines differs from ordinary levulose in being precipitated by basic lead acetate.

The presence of levulose in the urine of a diabetic does not necessarily mean that the patient's powers of assimilating that sugar are defective. It will be remembered that levulose can be artificially formed from dextrose by gently heating a faintly alkaline solution of the latter, and it would seem that a similar change sometimes takes place in the body. Such a *spurious levulosuria* may apparently occur when large quantities of alkali are being taken and the urine has an alkaline reaction. According to Koenigsfeld, it is also met with when there is reduced gastric acidity and increased intestinal alkalinity. The apparent diminution in the excretion of sugar that results from a course of treatment with alkaline mineral waters, with a return to the former level on the completion of the course, may possibly be explained in some such way ; for if the sugar is estimated throughout the treatment with the polariscope, or by titration, and the results are worked out on the assumption that dextrose alone is present, readings that are not really comparable will be obtained. The easiest way to guard against such an error is to check the results by fermentation.

The amount of dextrose present in cases of mixed dextrosuria and levulosuria varies very much. They may be conveniently divided into two classes—(a) those in which the dextrosuria is relatively slight, such as have been described by Zimmer, Czapeks, Röhmann, May, Lion, and Neubauer ; (b) those in which the levulose is associated with a considerable excess of dextrose, such as those reported by Rosin and Laband, Dub, Schleisinger, Schwarz, and Umber.

In either type the excretion of levulose has usually been found to be under 2 per cent., with a total output of 30 grams, or less, a day. The case described by Zimmer and Czapeks is, however, a striking exception, for as much as 4·4 per cent. of levulose, with a twenty-four hours' excretion of 176 grams, was there met with. On some days 5·4 per cent. of dextrose, equivalent to 216 grams in the twenty-four hours, was also passed.

Source of the Sugar.—The levulose present in the urine of some cases of levulosuria is apparently of alimentary origin, being derived from cane-sugar, honey, fruits, vegetables, &c., contained in the diet, for if such substances are excluded it disappears. Thus in Neubauer's case the levulosuria ceased in one or two days, and in the cases reported by Lépine and Schwarz after a somewhat longer

interval, when levulose-yielding foods were excluded.   In Seegen's
case, and also in Rosin's and Schleisinger's, the levulosuria
diminished, but did not quite disappear.   As ingested levulose
is apparently converted in the liver into glycogen, which subse-
quently breaks down into dextrose, it would seem that the failure
to assimilate levulose on the part of these patients is due to the
sugar not being converted into glycogen in the ordinary way.
Neubauer found that a definite proportion (15 to 17 per cent.)
of the levulose given by the mouth was excreted in the urine in
his case.   He therefore suggests that a certain proportion of the
levulose of the food is, under normal conditions, directly oxidised
without passing through the glycogen stage, and that a failure of
this oxidation may be the cause of levulosuria.   Feeding experi-
ments by other observers have not, however, given similar results.
They show that the greater part of the levulose administered is
retained within the organism, and only a small part is excreted in
the urine, also that the latter does not bear any definite relation to
the whole.   Inulin, like starch in mild cases of diabetes, appears to
be better tolerated than levulose, owing probably to its being only
slowly decomposed and absorbed from the intestine.

In some cases it has been found that when levulose was taken
by the mouth, even in doses calculated to exceed the limit of
tolerance of a normal person, no increase in the urinary levulose
has resulted.   Neubauer, for instance, has described a case in
which, although large doses of levulose caused no increase in the
output of that sugar, the administration of dextrose brought about
an increased excretion of both dextrose and levulose, suggesting
that the dextrose was in part converted into levulose within the
body and excreted as such in the urine.   That such a conversion
of one sugar into the other can occur within the organism is also
suggested by Zimmer's case, for it is not likely that the whole of
the 176 to 92 grams excreted in the urine could be entirely derived
as such from the food.   The administration of dextrose does not,
however, of necessity cause an increase in the excretion of levu-
lose in the urine.   Other observers have noticed that carbohydrate
foods containing no levulose caused an increased output of that
sugar.   Thus Seegen states that bread brought about this result
in his case, and Schwarz found that with one of his patients, whose
urine had been made sugar-free by diet, the use of grain-foods caused
both the dextrose and levulose to reappear.   That levulose can be
formed from dextrose within the body is also suggested by the
experiments of Hédon, who demonstrated levulose in the blood
of depancreatised dogs.   According to Schleisinger, injections of

phlorhidzin in cases of levulosuria only cause the appearance of dextrose in the urine.

Symptoms.—When levulose alone is present in the urine the symptoms are of a mild type, like those met with in cases of slight glucosuria. Polyuria is absent, the specific gravity of the urine is not high, the amount of sugar is small, and there is no thirst, wasting, or other characteristic sign. In one recorded case the levulose was discovered by accident in the urine of a patient suffering from transverse myelitis, in two obesity was a concomitant symptom, in some there has been a family history of diabetes, and in several nervousness and mental depression have been the most noticeable feature of the case. Since the presence of levulose in the urine appears to depend upon interference with the functions of the liver in many instances, it is possible that the last-named symptoms may be also dependent upon this, and arise from intestinal toxines having free access to the systematic circulation. Levulose in association with dextrose in the urine is not apparently accompanied by any particular symptoms, and may be met with in mild as well as in severe cases of diabetes. The significance of its presence is not certain, but it is probable that it indicates a functional derangement of the liver, which may be of a temporary or permanent character.

Detection.—In cases of pure levulosuria the recognition of the sugar is not a difficult matter. The urine gives the reduction tests with copper, bismuth, &c., is fermented by yeast, and forms with phenylhydrazin an osazone with the same melting-point as dextrosazone. It is, however, levo-rotatory, and gives Seliwanoff's reaction by either Rosin or Borchardat's modification. To exclude other levo-rotatory substances, the urine should be fermented with yeast and again examined with the polariscope. It is also advisable to control Seliwanoff's test by repeating it with the fermented urine to prove that it fails after the removal of the levulose. Finally, the levulose can be separated out as the methylphenylosazone, or as the calcium compound, and its properties investigated.

When the urine contains both levulose and dextrose Seliwanoff's reaction is a useful preliminary test, for when it is positive before, and negative after, fermentation it points to the presence of a ketose. If it is also found that the percentages of sugar shown by fermentation, or titration, and by polarisation of the urine are not the same it tends to confirm this conclusion, especially if the difference is marked.

The percentage of levulose and dextrose may be calculated from

the quantities of sugar found on fermentation and by the polariscope as follows :—

$$D + L = \text{`` } a \text{ ''} \text{ per cent. by fermentation.}$$
$$D - L = \text{`` } b \text{ ''} \text{ per cent. with the polariscope.}$$
$$2D \quad = (a + b)$$
$$\therefore D \quad = \frac{(a + b)}{2}, \text{ and } L = a - \left(\frac{a + b}{2}\right)$$

where $D = $ dextrose, and $L = $ levulose.

If the urine shows a levo-rotation `` c '' after fermentation, owing to the presence of beta-oxybutyric acid, &c., this must be allowed for :—

$$D + L = \text{`` } a \text{ ''} \text{ per cent. by fermentation.}$$
$$D - L = \text{`` } b \text{ ''} + \text{`` } c \text{ ''} \text{ per cent. on polarisation.}$$
$$2D \quad = (a + b + c)$$
$$\therefore D \quad = \frac{(a + b + c)}{2}, \text{ and } L = a - \left(\frac{a + b + c}{2}\right)$$

If the sugar is estimated by titration and polarisation, the percentage of levulose is found by dividing the difference between the percentages of sugar obtained by the two methods, by 2·69, provided that other levo-rotatory substances are absent, since 1 gram of levulose is equivalent in its reducing powers for Fehling's solution to 0·925 gram of dextrose, and a 1 per cent. solution of levulose turns the ray of polarised light $-0·93°$ to the left ; therefore a 1·76 per cent. solution is as strongly levo-rotatory as a 1 per cent. solution of dextrose is dextro-rotatory. Hence—

$$xD + y(0·93) = \text{`` } a \text{ ''} \text{ per cent. by reduction.}$$
$$xD - y(1·76) = \text{`` } b \text{ ''} \text{ per cent. by polarisation.}$$
$$y(0·93 + 1·76) = (a - b).$$
$$\therefore y = \frac{(a - b)}{2·69}$$

Should the percentages obtained on titration and on polarisation be approximately equal, it is probable that some other levo-rotatory substance besides levulose is present. In this case, or when the presence of such bodies is suspected for other reasons, the optical activity of the urine after complete fermentation must be determined, and allowed for as shown above.

The levulose may be isolated as the methylphenylosazone, according to the method of Neuberg and Strauss :—

The urine is made acid by adding a few drops of acetic acid, boiled, and filtered. It is then evaporated to a syrup at 40° C., mixed with half its volume of 98 per cent. alcohol, heated for five minutes, cooled, and filtered. If the residue still possesses reducing powers the treatment with alcohol is repeated once or twice. The alcoholic extracts are filtered from any flocculent precipitate that may have formed, and decolorised with animal charcoal. The quantity of sugar present is then determined by titrating a sample. The remainder is evaporated

to a small bulk (30 c.c.), and mixed with methylphenylhydrazin, allow-ing 3 molecules for each molecule of sugar. After standing for one hour, any precipitate that has formed is filtered off, and the filtrate mixed with an equal volume of 50 per cent. acetic acid, and sufficient alcohol to give a clear solution. The mixture is heated in a boiling water bath for three to five minutes, or left at 40° C. for twenty-four hours. If a considerable amount of levulose is present the methyl-phenylosazone crystals separate out directly, or on adding a few drops of water. If only a small amount is present an oily precipitate forms. The osazone can be separated from this in a crystalline form by cooling and treating it with carbon dioxide and ether. The crystals are purified by recrystallising them from absolute alcohol in the cold, and may be further purified by dissolving in hot water, to which a little pyridin has been added. The solution is boiled with animal charcoal, filtered, and the crystals separated out. Methylphenyl-levulosazone appears as fine yellow crystals with a melting-point of 158° to 160° C. A solu-tion (0·2 gram) in pyridin-alcohol (4 grams pyridin, 6 grams absolute alcohol) is dextro-rotatory ($+1°$ 40′).

Maltosuria.—When maltose is present in the urine it is nearly always associated with dextrose, although a few examples of pure maltosuria have been described. No case of maltosuria appears to have been reported previous to the year 1888, when Le Nobel stated that he had found maltose in a urine examined by him. The next year another case was described by v. Ackeren, and subsequently others were reported by Rosenheim and Flatow, Charin and Brocard, Lépine and Boulud, Kottmann, Geelmuyden, Rosenberger, and Magnus-Levy. In the earlier cases the diagnosis was based entirely on the difference between the results obtained on titration and on examining the urine with the polariscope, and on the alteration produced by heating with hydrochloric acid; but as the differences observed were always very slight, and might be accounted for by experimental errors and in other ways, the true significance of the results obtained in this way is doubtful. Later observers have separated the phenylosazone and based their conclusions mainly on its melting-point (202° to 208° C.), its nitrogen content (10·6 per cent.), its solubilities, and the effects of a solution in pyridin-alcohol on polarised light ($+1°$ 30′).

In most of the recorded cases of maltosuria the calculated amount of maltose has been under 0·5 per cent. Magnus-Levy states that he met with 1·5 per cent. in association with 2 per cent. of dextrose in one case, but that the maltosuria only lasted for two days, and appeared to be due to the consumption of a quantity of beer by the patient.

Maltose was discovered by Geelmuyden in the urines of seven

out of nine cases of diabetes by means of a special method of separating the osazone that he employed. According to Lépine and Boulud, a small quantity of maltose is not rarely present in the urine of diabetics when they first come under observation. This they consider is in part derived from the food, as it frequently disappears when the patient is put on a strict diet. In some instances, however, the maltosuria persists even when the patient is taking only nitrogenous and fatty foods, so that it must also have some other source of origin. Since they found from 0·2 to 0·3 per cent. of maltose in the urine of depancreatised dogs, which had been kept on a purely nitrogenous diet for some time prior to the operation, Lépine and Boulud suggest that the presence of this sugar in the urine of diabetics may be due to imperfect transformation of glycogen consequent on disease of the pancreas. It is true that maltose has been detected in the urine during life in cases in which disease of the pancreas was found post-mortem, but they are too few to prove that there is any causal connection between the two. Rosenheim's case passed 0·1 to 0·5 per cent. of maltose in his urine, had fatty stools, lost thirty pounds in weight in nine months, and after death interstitial pancreatitis was found. In Kottmann's case of diabetes with maltosuria, atrophy of the pancreas was discovered post-mortem. I have had the opportunity of examining specimens of urine from a large number of cases of typical pancreatic disease, over two thousand, and I have only met with two in which an osazone having the characters of maltosazone was obtained in sufficient quantity for an accurate investigation, and five in which a small deposit of crystals, probably also maltosazone, was given. One of the former was a patient in whom an operation for stone in the common duct with chronic pancreatis had been performed four years previously.

Maltosuria has been observed by Charin and Brocard in lying-in women, and Rosenberger met with a sugar resembling maltose in a case of croupous pneumonia.

Detection.—Maltose reduces alkaline solutions of copper and bismuth, is strongly dextro-rotatory ($+137°$), and is easily fermented by yeast. Its detection in the urine is usually based upon the difference obtained on examining with the polariscope and by titration. The optical activity of maltose is about two and a half times greater than that of dextrose, while its reducing power is only about two-thirds as great. As other substances, such as pentoses, lactose, glucuronic acid compounds, oxybutyric acid, and amino acids can also cause a difference in the readings, it is only when the urine contains a considerable quantity of maltose that a

satisfactory diagnosis can be made in this way. Further evidence can be obtained by preparing the phenylosazone, which is characterised by its solubility in hot water, its melting-point of 202° to 208° C., and its optical activities. Its solution in alcohol is dextro-rotatory, the pyridin-alcohol solution is also dextro-rotatory (+ 1° 30'), but its solution in glacial acetic acid is levo-rotatory.

Isomaltose.—Isomaltose is stated to have been recovered from normal urine in small quantities by Baisch, Lemaire, Porcher, and Reinbold by the benzoyling process, and by Pavy and Siau as isomaltosazone.

The question as to whether it exists preformed in the urine, or is derived from dextrose in the process of separation, has not yet been settled, even by those who believe in its existence, and some observers deny that it does. Mayer points out that the reactions described as characteristic of isomaltose are also given by glucuronic acid, and that to depend upon the melting-point of the osazone as the chief distinguishing feature, as some observers have done, is most unsatisfactory. According to Cremer an alimentary isomaltosuria is possible, and it may be that the traces met with in some urines are of intestinal origin. Wöhl and others have shown that isomaltose is very readily formed in small quantities by digesting dextrose with dilute hydrochloric acid. Rosin and Alfthan have found isomaltose in diabetic urines, and it is considered by Pavy and Siau that it is to the presence of this substance that the increased reduction, shown by some diabetic urines after heating with an acid, is due.

Detection.—Isomaltose, like maltose, reduces alkaline solutions of copper and bismuth, but while maltose is fermented by yeast, isomaltose is not. They are most readily distinguished by the characters of their osazones, that of isomaltose melting at 150° to 153° C., while maltosazone melts at 202° to 208° C.; moreover, isomaltosazone can be obtained from the urine after any dextrose, levulose, or maltose that may be present has been removed by fermentation.

Laiose.—This sugar was isolated by Leo from the urines of three out of twenty-one diabetics. Its presence was inferred from the quantitative estimation of dextrose by titration, being 1·2 to 1·0 per cent. more than was shown by the polariscope. In one case the urine was optically inactive, and was found on titration to contain 1·8 per cent. of dextrose. Leo subsequently isolated the sugar and investigated its properties. It is distinguished from other sugars by its salty, rather than sweet, taste, its slight reducing

powers as compared with dextrose (0·4 : 1), by being unfermented by yeast, by being levo-rotatory (– 26° 7′), and by its forming with phenylhydrazin a yellowish-brown, non-crystalline, oil, that is insoluble in water, but is soluble in alcohol. It has been variously regarded as a hexose and as a pentose (d-xylose ?).

Heptose.—From the urine of one case of diabetes Rosenberger separated a sugar which in many respects resembled laiose, but was considered by him to be a heptose. The isolated sugar was obtained as a brown fluid which reduced alkaline solutions of copper, and formed with phenylhydrazin an osazone with a melting-point of 195° C., corresponding to that of a heptose. A solution of the osazone in pyridin was optically inactive. The urine from which the sugar was derived was levo-rotatory, but the sugar itself was found to be optically inactive.

Paidose.—Under this name Geelmuyden described a sugar that he found in the urine of diabetic children. It was optically inactive, or only very feebly active, slowly reduced Fehling's solution, and yielded an osazone with a melting-point of 175° to 190° C. The orcin and phloroglucin reactions were negative.

Pentoses.—Some diabetic urines contain traces of a pentose, but this question will be more fully dealt with when chronic pentosuria is considered (see mixed pentosuria and dextrosuria, p. 396).

Glycogen (Erythro-dextrin).—Reichardt found that the urines of several diabetics that he examined after the complete, or almost complete, disappearance of the sugar still reduced alkaline solutions of copper on prolonged boiling. This he attributed to the presence of a dextrin-like substance that was coloured reddish-brown by iodine, which he isolated from the urines. Leube obtained a similar substance from the urines of two cases of diabetes, and considered it was glycogen. He was unable to find it in the urines of healthy people, or those suffering from diabetes insipidus. The exact nature of this body is not certain, but on physiological grounds it is more likely to be glycogen than erythro-dextrin.

Animal Gum (**Landwehr**).—This, which is probably not a single substance but a mixture, is said to occur in normal urines in quantities of 0·1 to 0·2 grams daily. Alfthan found that it was increased in diabetes mellitus, as much as 1·2 to 36·9 grams being excreted in the twenty-four hours. It is slightly dextro-rotatory, is not fermented by yeast, is not coloured by iodine, and gives with copper a precipitate that is insoluble in alkalies and does not darken on boiling.

Inosite.—Inosite has the same empirical formula as the hexoses ($C_6H_{12}O_6$), but it belongs to the aromatic series, being hexahydroxybenzol, and is not, as was at one time thought, a carbohydrate. It is, however, convenient to consider it here.

It was said by Neukomm, Cloetta, Gallois, and Külz that inosite is not present in normal urine in demonstrable amounts, but Rosenberger and Starkenstein found it in every urine they examined in quantities up to about 0·08 grams in the twenty-four hours. According to Strauss excessive water drinking, with consequent polyuria, gives rise to a varying degree of inosituria.

Inosite has been found in the urine in increased quantities in three pathological conditions—namely, diabetes insipidus (Vohl, Strauss, and Külz), nephritis (Cloetta and Külz), and diabetes mellitus (Vohl, Gallois, Külz, and Lava). In the latter condition it is not constantly present, and was only found by Külz and Lava five times in thirty cases, and in all of these there was marked polyuria.

Detection.—Inosite does not reduce alkaline solutions of copper and bismuth. It is precipitated by lead acetate, is optically inactive, and does not ferment with yeast ; it is, however, decomposed by *B. lactis* with the formation of lactic acid, and subsequently yields butyric acid. It does not form an osazone with phenylhydrazin. It may be isolated and recognised as follows :—

Cooper-Lane Method.—Any albumen that may be present is removed by boiling and filtering. The phosphates are then precipitated out with baryta water, and the filtrate, after being heated, is treated with lead acetate, avoiding an excess. The mixture is allowed to stand for some time, and the precipitate that has formed is then filtered off, washed, suspended in water, decomposed with sulphuretted hydrogen, filtered, and after standing for some time to allow the uric acid to separate, the filtrate is evaporated to a small bulk. The creatinin is removed by mixing the residue with one to four volumes of alcohol, and boiling. If a heavy precipitate which sticks to the glass forms, the clear, hot, alcoholic fluid is simply decanted, but if it separates out in flocculi it is filtered hot through a warm filter, and then allowed to cool. The fluid is then left to stand for twenty-four hours. If inosite is present in appreciable quantity it will separate out in crystals which may be filtered off and washed with cold alcohol. If no crystals appear, the inosite may be separated in mother-of-pearl plates by adding ether, little by little, avoiding an excess, to the clear alcoholic solution until a slight milkiness, that does not disappear, results, and leaving in the cold for twenty-four hours.

The crystals of inosite are rhombohedral in form and melt at 225° C. They are soluble in water (1 : 75), but are insoluble in alcohol and ether. They may be recognised by the following tests :—

1. *Scherer's Test.*—A small quantity of the precipitate is mixed with nitric acid on platinum foil, and evaporated almost to dryness. To the residue are added a little ammonia and a drop of calcium chloride solution, and the evaporation continued to dryness. If inosite is present a beautiful rose colour results. Unless the crystals are fairly pure a typical reaction is not obtained.

2. *Seidel's Test.*—This test is carried out in the same way as the preceding, except that strontium acetate is used instead of calcium chloride. It gives a green colour with a violet precipitate. A positive reaction is obtained with 0·3 mg. of inosite (Fick).

BIBLIOGRAPHY

LEVULOSE

Borchardat, *Zeit. f. physiol. Chem.*, 1908 ; *Münch. med. Woch.*, 1909.
Carles, *Chem. Zentralb.*, 1890.
Cotton, *Bull. d. Soc. Chem.*, 1880.
Czapeks, *Prager med. Woch.*, 1876.
Dub, *Dissertation*, Leipzig, 1902.
Graul, *Centralb. f. inn. Med.*, 1905.
Haycraft, *Zeit. f. physiol. Chem.*, 1894.
Koenigsfeld, *Zeit. f. klin. Med.*, 1909.
Külz, *Zeit. f. Biol.*, 1884, 1890.
Lépine and Boulud, *Rev. d. Méd.*, 1904.
Lion, *Münch. med. Woch.*, 1903.
Marie and Robinson, *Bull. Soc. méd. d. hôp. d. Paris*, 1897 ; *Semaine Méd.*, 1897.
May, *Arch. f. klin. Med.*, 1896.
Moraczewski, *Zeit. f. klin. Med.*, 1907.
Moritz, *Centralb. f. inn. Med.*, 1891.
Neubauer, *Münch. med. Woch.*, 1905.
Neuberg and Strauss, *Zeit. f. phys. Chem.*, 1902.
Personne and Henniger, *Bull. d. Soc. d. Chem.*, 1880.
Röhmann, *Centralb. f. inn. Med.*, 1884.
Rosin and Laband, *Zeit. f. klin. Med.*, 1902 ; *Ber. d. Deut. Chem. Gesellsch.*, 1902.
Schleisinger, *Arch. f. exp. Path.*, 1903.
Schwarz, *Arch. f. klin. Med.*, 1903.
Seegen, *Centralb. f. d. med. Wiss.*, 1884.
Strauss, *Deut. med. Woch.*, 1901.
Umber, *Salkowski's Festschr.*, 1904.
Ventzke, *Journ. f. prakt. Chem.*, 1842.
Zimmer, *Deut. med. Woch.*, 1876.

MALTOSE

Ackeren, *Berl. klin. Woch.*, 1889.
Charin and Brocard, *Compt. Rend. d. Soc. d. Biol.*, 1898.

Geelmuyden, *Zeit. f. klin. Med.*, 1905.
Kottmann, *Dissertation*, Geneva, 1901.
Lépine and Boulud, *Compt. Rend. d. Acad. d. Sci.*, 1901.
Levy, v. Noorden's *Handb. d. Path. u. Stoffwech.*, 1907.
Le Nobel, *Arch. f. klin. Med.*, 1888.
Rosenberger, *Deut. med. Woch.*, 1906.
Rosenheim and Flatow, *Berl. klin. Woch.*, 1898.

ISOMALTOSE

Alfthan, *Deut. med. Woch.*, 1900.
Baisch, *Zeit. f. phys. Chem.*, 1894.
Cremer, *Zeit. f. phys. Chem.*, 1892.
Lemaire, *Zeit. f. phys. Chem.*, 1895.
Mayer, *Zeit. f. phys. Chem.*, 1901.
Pavy and Siau, *Journ. of Physiol.*, 1901.
Porcher, *Chem. Zeit.*, 1902.
Reinbold, *Pflüger's Arch.*, 1902.
Rosin, *Deut. med. Woch.*, 1900.
Wöhl, *Ber. d. Chem. Gesellsch.*, 1890.

LAIOSE

Fischer, *Lieb. Ann.*, 1892.
Leo, *Virchow's Arch.*, 1887.

HEPTOSE

Rosenberger, *Zeit. f. phys. Chem.*, 1906.

PAIDOSE

Geelmuyden, *Jahresber. f. Tiersch.*, 1903.

GLYCOGEN

Kotake, *Zeit. f. phys. Chem.*, 1910.
Leube, *Virchow's Arch.*, 1888.
Reichardt, *Arch. d. Pharm.*, 1874.

ANIMAL GUM

Alfthan, *Dissertation*, Helsingfors, 1900, 1904.
Baisch, *Zeit. f. phys. Chem.*, 1894.
Freund, *Zentralb. f. Physiol.*, 1892.
Landwehr, *Zeit. f. phys. Chem.*, 1882–3–4–5; *Centralb. f. d. Med. Wiss.*, 1885; *Pflüger's Arch.*, 1886–7.
Lemaire, *Zeit. f. phys. Chem.*, 1895.
Mörner, *Skandin. Arch.*, vi.
Reinbold, *Pflüger's Arch.*, 1902.
Salkowski, *Berl. klin. Woch.*, 1905.
Wedenski, *Zeit. f. phys. Chem.*, 1888.

INOSITE

Cloetta, *Lieb. Ann.*, 1856.
Cooper-Lane, *Ann. Chem. Pharm.*, 1861.
Fick, *Chem. Zentralb.*, 1887.
Gallois, *De l'Inositurie*, 1864.
Külz, *Maly's Jahresb.*, 1875–6.
Lava, *Arch. f. klin. Med.*, 1891.
Maquenne, *Bull. d. Soc. Chem.*, 1887.
Neukomm, *Dissertation*, Zürich, 1859.
Rosenberger, *Münch. med. Woch.*, 1908.
Scherer, *Ann. d. Chem. u. Pharm.*, lxxxi.
Starkenstein, *Zeit. f. exp. Path. u. Therap.*, 1908.
Strauss, *Centralb. f. inn. Med.*, 1872.
Vohl, *Arch. phys. Heilk.*, 1858.

Lactosuria.—Next to dextrose the commonest sugar met with in the urine is lactose or milk-sugar. It is usually found in women in connection with pregnancy, or during lactation, but may also occur as an alimentary lactosuria under other conditions.

Alimentary Lactosuria.—The assimilation limit of a healthy adult for milk-sugar is stated by Worm-Müller to be 100 grams, but Halász has given 150 grams without producing lactosuria. For healthy children the limit of tolerance is, according to Grósz, 8·6 grams per kilo of body-weight. Occasionally 100 grams of lactose, taken in one dose, will cause the appearance of sugar in the urine, and a few cannot take 80, or even 50, grams of milk-sugar without excreting same. This does not indicate any disorder of metabolism, but depends upon a defect in the lactose-splitting ferment of the intestine, which allows a certain proportion of unaltered milk-sugar to be absorbed into the blood, whence it is excreted into the urine, for lactose cannot be converted into glycogen until it has been inverted, and this inversion can only take place before, or during, absorption from the intestine.

It has been found that in some diseases of the gastro-intestinal tract the assimilation limit for lactose is considerably lowered, Grósz met with 2 per cent. of milk-sugar in the urine of twenty-two out of twenty-three cases with gastric disorders, mostly carcinoma with dilatation, when they were given 150 grams of lactose fasting. Méhu states that lactose is sometimes found in the urine of the patients who have taken large quantities of milk over considerable periods. Grósz, and later Langstein and Steinitz, showed that the reducing substance found in the urines of infants suffering from gastro-intestinal disorders is lactose. The latter, also Meyer and Zuelzer, have met with galactose under the same conditions in some instances, the monosaccharide constituting the bulk of the sugar in one instance. According to Grósz, the assimilation limit in these cases may be as low as 2·0 to 2·9 grams per kilo. Lying-in women appear to have a lowered assimilation limit for lactose,

as well as for dextrose and levulose, 100 grams, and in some even 50 grams, causing slight lactosuria. Zuelzer and Hess state that lactosuria can also be produced after abortion, and in parturient women, by administering 150 grams of dextrose.

Lactose, taken as such, or in the form of milk, by diabetics is not excreted unchanged but appears as dextrose, the proportion varying in different cases. In the mild type a part, or even the whole, is often made use of, but in severe cases an equivalent amount of dextrose is passed in the urine. Thus, in a case reported by Borchardat and Finkelstein, 100 grams of milk-sugar given by the mouth were entirely excreted as dextrose. Bourquelot and Troisier, and subsequently Voit, found lactose in the urines of diabetics who had consumed a large amount of milk, but only in very small quantities.

Spontaneous lactosuria is confined to women, and only occurs during the later months of pregnancy and after childbirth. As far back as 1849 it was noticed by Heller that a reducing substance may be met with in the urine of women during the period of lactation, and in 1877 it was definitely proved by Hofmeister and Kaltenbach that this substance is milk-sugar. Lactosuria is a much more common phenomenon in nursing women than is generally supposed, and milk-sugar is probably seldom entirely absent from the urine at some time or other during that period. It is most generally met with during the first few days after labour, but may be found as late as six months subsequently. According to MacCann the commonest time is between the fourth and fifth day of the puerperium. Ney found lactosuria in 115 out of 148 (77 per cent.) parturient women that he examined, but only in 16 per cent. of those who were pregnant. Gérard also found evidence of temporary lactosuria in only five out of forty-one (12 per cent.) pregnant women approaching term, while Lemaire detected lactose in the urine of eighteen out of nineteen lying-in women under his care. Blumenthal states that some 80 per cent. of women who nurse their children have lactosuria, but that only some 20 per cent. of suckling mothers pass milk-sugar in their urine during the period that the child is being fed, when, however, suckling is suddenly stopped, or the breasts become engorged, or inflamed milk-sugar generally makes its appearance in the urine.

As a rule the lactosuria is of short duration, but occasionally it lasts for some time, persisting for as long as five months in a case reported by Pavy. The quantity of sugar excreted is usually small, seldom exceeding 1 per cent., and there is no polyuria. The total daily output is generally under 10 to 20 grams. Naunyn,

however, met with cases in which the urine contained 2 to 3 per cent.
of lactose, Lemaire found 4 per cent., and Porcher has reported
cases with as much as 7 per cent. The administration of dextrose
increases the excretion of milk-sugar. Hess found that after
administering 150 grams of dextrose, from 8·4 to 19·5 grams of
lactose appeared in the urine.

The milk-sugar excreted in the urine in spontaneous lactosuria
is formed in the mammary glands. Porcher has shown that if
these organs are removed in cows and goats immediately before
parturition, lactosuria does not occur, but that a glycosuria of
variable intensity rapidly sets in and lasts about twenty-four hours.
A similar result follows amputation of the breasts in lactating
animals, and is associated with hyperglycæmia, showing that in
the absence of the mammary glands dextrose is not converted into
lactose but is excreted in the urine.

Diagnosis.—It is most important that lactosuria should be
carefully distinguished from glycosuria, since the former is temporary
and does not indicate a serious disturbance of metabolism, whereas
the latter, as we have seen, is of very grave significance in pregnant
and parturient women, and demands prompt attention. The com-
plete identification of lactose in the urine necessitates its isolation
by a lengthy process, but for clinical work this is not usually neces-
sary, as its presence can generally be established with a reasonable
degree of probability by considering the clinical characters of the
case in conjunction with the results of a series of tests applied
directly to the urine.

Whether the urine contains lactose or glucose it will reduce
alkaline solutions of copper and bismuth, but with lactose the re-
duction is not as prompt as it is with dextrose, and does not take
place until the mixture is boiled. When lactose alone is present
the fermentation test will be negative during the first twenty-four
hours, and since phenyl-lactosazone does not readily crystallise out
except from pure solutions of the sugar, the phenylhydrazin test
will not yield a definite crystalline precipitate. If, however, the
urine is boiled with 5 per cent. sulphuric acid for a short time,
neutralised with ammonia, and then treated with phenylhydrazin,
it will give crystals of glucosazone and galactosazone. The
reducing power of the urine will also be found to be increased by
boiling with a mineral acid and neutralising, but it is unchanged
after boiling with citric acid. Treatment with sulphuric acid does
not usually affect the result of the fermentation test, as it might be
expected to do theoretically, since the large amount of sulphate
formed interferes with the growth of the yeast. Voit's, or Buchner's,

modification of Rubner's test, and the Wöhlk (Malfatti) reaction may also be employed to confirm the results obtained by these means. Lactosuria can be distinguished from pentosuria by the phloroglucin, and orcin tests, &c.

Before leaving the subject one other cause of the presence of lactose in the urine must be mentioned, namely, malingering. Hysterical persons, especially women, soldiers, and others, have been known to inject milk into the bladder, or to mix it with their urine after it has been passed, with a view to simulating diabetes or chyluria. If the possibility of such a deception is borne in mind it is not difficult to detect. In a case that came under my observation condensed milk was mixed with the urine, and the fraud was exposed by discovering both lactose and cane-sugar, as well as by the microscopical characters of the emulsion.

Galactosuria.—In healthy people, according to Bauer, 20 grams of galactose taken by the mouth do not give rise to galactosuria ; after 40 grams about 1 gram may be excreted in the urine unchanged, but with 100 grams galactosuria always results. When there is cirrhosis of the liver, galactose is not as well tolerated as in health, 1 gram appearing unchanged in the urine after the ingestion of 20 grams, and 4 grams after 40 grams have been taken. In mild cases of diabetes no variation from the conditions present in health was observed, but in severe cases the ingestion of 40 grams, although it did not give rise to galactosuria, increased the output of dextrose, and 100 grams caused both an increase in the excretion of dextrose and galactosuria. With the exception of the discovery by Langstein and Steinitz, already referred to, that the sugar noticed by Grósz to be present in the urines of children suffering from gastrointestinal catarrhs is often a mixture of lactose and galactose, in which the latter may predominate, no observations on spontaneous galactosuria appear to be recorded.

The presence of galactose in the urine is recognised by the results of the reduction tests, a comparison of the effects on polarised light, before and after treatment with dilute acids, the melting-point of the osazone (194° C.), the formation of mucic acid, and the results of the fermentation test. The latter is usually negative for the first six hours with ordinary yeast, but later gas-formation may occur.

Saccharosuria.—The assimilation limit for cane-sugar is so high that it rarely passes into the urine unchanged in healthy individuals. Occasionally, however, traces may be met with in the urine of patients suffering from gastro-intestinal disorders, and

especially in children. Smolenski has reported a case in which the ingestion of cane-sugar was followed by a marked output in the urine, which gave a " Cammidge " reaction.

The recognition of cane-sugar is chiefly important from its occasional use by malingerers to simulate diabetes, the sugar being directly added to the urine. In such cases the urine will have a high specific gravity, give an atypical reduction when boiled with alkaline solution of copper, ferment but slowly, and give only a few, or no, crystals with phenylhydrazin as in Brown's case. If the urine is concentrated, boiled with hydrochloric acid for half an hour, neutralised with sodium carbonate, and re-examined, it will give the typical tests for dextrose, and levulose, and will be found to be levo-rotatory whereas it was formerly dextro-rotatory. It is stated by Hirschberg that cane-sugar can be differentiated from dextrose, pentoses, &c., by the following procedure. The suspected liquid, if sterile, is placed in an incubator for twenty-four hours, or if in a hurry or contamination is feared is boiled for forty-five minutes, with an equal quantity of deci-normal sodium hydrate solution. Dilute solutions of sodium hydroxide readily affect dextrose, mannite, maltose, mannose, lactose, levulose, galactose, and invert sugar, complete decomposition occurring under the circumstances described, but saccharose is unaffected, and may be easily detected with the polariscope.

Pentosuria.—Cases of pentosuria, like those of levulosuria, can be divided into three classes—(1) an alimentary type, (2) a pure spontaneous form, (3) a mixed type, in which the pentose is associated with dextrose. There is, however, this important difference that, while the levulose is in all cases the same, the pentose found in pentosuria is a different form of 5-carbon atom sugar according to the circumstances that determine its excretion.

1. *Alimentary Pentosuria.*—The assimilation limit of the healthy organism has been proved experimentally to be much lower for pentoses than it is for the hexoses ; very small doses, 0·25 gram of arabinose, 0·05 gram of xylose, according to Ebstein, 1·0 gram of rhamnose, according to Cremer, causing a previously pentose-free urine to give a colour reaction for the sugar. The proportion that is excreted unchanged in the urine appears to vary considerably, but it is not unlikely that this depends more upon the condition of the alimentary tract and the amount that is destroyed there before the sugar is absorbed, than upon individual variations in assimilative power.

Since many vegetable foodstuffs contain pentosans, and some,

such as cherries, plums, strawberries, whortle-berries, fruit juices, &c., are comparatively rich in such substances, it is not surprising to find that a number of observers, including Blumenthal, Barczewski, v. Jaksch, and Johnstone, have detected a pentose in the urine of persons who have consumed considerable quantities of these sub- stances, and that such cases are most common in the summer when fruit, vegetables, and fruit juices are largely taken. Johnstone produced alimentary pentosuria in sixteen out of eighteen persons by giving them from a half to a litre and a half of apple juice, and found that the effect might persist for several days when the larger quantities were consumed. According to this observer the ad- ministration of morphia increases the amount of pentose excreted.

The sugar found in the urine in alimentary pentosuria is optically active, being the l-arabinose contained in the fruits, &c., so that the urine is dextro-rotatory. The quantity excreted is always small, but sufficient is present to give a reduction test which may prove misleading. It is, therefore, important to bear in mind that a slight, or doubtful, reduction may be due to an alimentary pentos- uria, especially when the patient is a vegetarian, in localities where fruit juices, &c., are extensively used, and in the summer time.

It is often stated that the urines of herbivorous animals usually give a pentose reaction, and that this is due to the pentosans con- tained in their food, since hay, for example, contains over 21 per cent. According to experiments made by Cominotti, the greater part of the pentose taken by a fasting horse is utilised by the organism and only a comparatively small proportion passes in the urine. Small quantities reappear on prolonging the inanition.

Both with animal and human urines a diagnosis of pentosuria should not be made, as is sometimes done, merely on the results of the colour tests for furfurol, since these may also be given by glucuronic acid compounds. Ebstein examined twenty-two ap- parently normal human urines with the phloroglucin test and ob- tained a positive reaction with fourteen, Cremer states that almost every urine gives a more or less marked reaction, and Funaro came to the same conclusion. Similar results are also obtained with the orcin test, unless it is very carefully carried out. Statements that pentoses appear in the urine as a result of the administra- tion of drugs, foods, &c., must therefore be accepted with reserve, unless the proof rests on a more sure foundation than a mere application of these tests.

2. *Spontaneous, or essential, pentosuria* is quite a different con- dition to the alimentary form, having no relation to food, and persisting when pentose-containing substances are excluded from

the diet. Moreover, the sugar excreted in every case so far, with the possible exceptions of those described by Luzzato, and Elliott and Raper, has apparently been optically inactive arabinose, a substance that is not met with otherwise in the animal or vegetable kingdoms.

The first case of essential pentosuria was described by Salkowski and Jastrowitz in 1892. The patient was a young man, a victim of the morphia habit and suffering from neurasthenia. When he first came under observation his urine contained traces of dextrose, but after the morphia was discontinued this disappeared. It was then found that it gave the reduction tests for sugar, but did not ferment with yeast, was optically inactive, and yielded with phenyl-hydrazin an osazone having a melting-point of 159° C. The melting-point of the osazone suggested that the sugar was a pentose, and further investigation confirmed this conjecture. Since 1892 other cases of essential pentosuria have been described by Blumenthal, Reale, Colombini, Bial, Meyer, Romme, Brat, Luzzato, d'Amato, Bendix, Klercher, Adler, Tintemann, Schüler, Johnstone, v. Jaksch, Kraft, Blum, Janeway, Kaplan, Rosenfeld, Cassiver and Bamberger, Chobola, Vas, Jolles, Wall, Elliott and Raper, so that some thirty-eight or forty are now on record. Several of these, including the cases reported by Reale, Colombini, d'Amato, and Kaplan, are probably not to be regarded as cases of true essential pentosuria, but were most likely of the alimentary type.

Chronic pentosuria is a rare condition. Jolles, in an examination of 3000 normal and pathological urines in the course of two years, only met with four undoubted cases, and in over 4000 urinary analyses that I have made during the past seven years I have not met with a single example, although pentoses have been systematically tested for. So far the great majority of the recorded cases have been met with in Germany, especially at watering-places to which patients with the milder forms of diabetes are accustomed to resort, and it is noteworthy that the American cases have been of German or Russian descent. A striking proportion of the cases so far met with have been Jews.

The amount of pentose present in the urine has always been small, very rarely exceeding 1 per cent., and generally being under that amount. In many cases the total daily excretion is not stated, but, in those where the quantity of urine passed is given, it works out at under 10 grams a day, and usually considerably less. The quantity appears, however, to vary from time to time. In a case investigated by Blumenthal, for instance, 7 grams were passed in the twenty-four hours, but two years later an analyses by the same method (Knapp) showed only about 3 to 4 grams.

The urine is acid in reaction and the specific gravity varies from 1·025 to 1·035. Fehling's solution is reduced as it is by urines containing ½ per cent. or so of dextrose—that is to say, only on boiling the mixture. The sudden, delayed reduction, described by some observers as characteristic of pentose-containing urines, is attributed by Bial to the specimens having been kept for some time by means of a preservative. Pentoses are not fermented by yeast, so that the reducing power of the urine is not impaired by being incubated with yeast for twenty-four hours. This affords a ready means of differentiating the sugar when it occurs in a urine along with dextrose. It forms with phenylhydrazin a crystalline osazone which is soluble in hot water, and, after re-crystallising, melts at 156° to 160° C. The osazone yields about 17 per cent. of nitrogen. The urine in essential pentosuria is optically inactive, unless dextrose is also present, and gives the usual colour reactions for pentoses. Of these Bial's modification of the orcin test is the most useful clinically. According to Klercher, there is a certain parallelism between the amount of pentose excreted and the total nitrogen content of the urine, but the latter is not notably increased, nor is the output of purin bodies, or phosphorus, in any way abnormal.

The ordinary method of estimating sugars in the urine with Fehling's solution cannot be employed satisfactorily for the pentoses, since the cuprous hydrate does not separate out well. Even when Knapp's, or Allihn's, method is used, or the phloroglucin precipitate is weighed, the results, according to Neuberg, are too low, since much of the pentose is in combination with urea and does not reduce until the ureide is broken down by heating with an acid. Neuberg mentions 30 to 36 grams as the total amount of pentose that may be excreted in a day if the sugar combined with urea is taken into account.

Symptoms.—Chronic pentosuria does not give rise to any particular train of symptoms. Polyuria, thirst, excessive hunger, wasting, and the other characteristic symptoms of chronic dextrosuria have not been present in any of the reported cases. Only in one, that of Colombini, was there any affection that is usually associated with diabetes. This patient was an Italian, aged fifty, and appeared to be suffering from xanthoma diabeticorum. When he was treated with arsenic, and put on milk and meat in place of his previous vegetarian diet, the skin condition improved and the pentose disappeared from the urine. As the pentosuria was a transitory condition occurring in a vegetarian, and the optical characters of the urine were apparently not investigated, it is probable that

the case was really of the alimentary type, and it is not certain that there was any connection between the skin condition and the presence of the sugar in the urine.

The etiology of chronic pentosuria is not clear. The fact that Salkowski and Jastrowitz's patient, and also Reale's, were morphia habitués, and that one of Bial's cases had the cocaine habit, has suggested that the condition might be dependent upon the abuse of drugs, but this has not been established in other cases. The presence of neurasthenic symptoms and neuralgic pains, in some cases (Salkowski and Jastrowitz, Cassiver and Bamberger), might point to a nervous origin, but this too has not been proved in other cases. It has been suggested that chronic pentosuria might be dependent upon some lesion or abnormality of the pancreas, but there is no evidence whatever to support this view. In the only case of pentosuria so far examined post-mortem the pancreas was unfortunately not closely investigated, but no gross pathological change was observed in it, or in any other organ, to account for the sugar in the urine (Blumenthal). In some instances the sugar has been found in the urine of apparently healthy individuals in the course of routine examination for life insurance, or for some other purpose. Some pentosurics have been members of diabetic families. Klercher's patients were brothers, and their father and another brother had died of diabetes. Schüler's patient had a brother and two sisters who were diabetic, and in one of the cases described by Rosenfeld the patient's father and brother had died of diabetes. This patient developed pentosuria after being in a railway accident. Such cases would seem to suggest that there is a relationship between pentosuria and diabetes, but there can be no doubt that, from a metabolic standpoint, pentosuria and glycosuria are quite distinct. It has been shown that the tolerance of pentosurics for dextrose is not in any way diminished, and that glucose only appears in the urine when taken in doses sufficient to overtax the assimilative powers of a normal individual.

The most striking feature about chronic pentosuria is its tendency to occur in several collaterals of a family. Brat's cases were a sixty-two-year-old woman and her fifty-year-old brother. The former had been treated for eight years as a diabetic before the true state of affairs was discovered, the latter was apparently quite healthy. Two of Blumenthal's cases were sisters. Of three cases described by Bial two were sisters, and the third their brother. Klercher's patients, who were brothers, have been mentioned. Janeway's cases were also brothers. There is, as yet, no record of an instance where chronic pentosuria has been

transmitted from parent to child, and nothing is known of con-
sanguinity of the parents of subjects of this condition, but the
tendency at the present time is to regard it as a congenital
abnormality of the chemistry of the body, or, as Garrod terms
it, " an inborn error of metabolism," like albinism, alkaptonuria,
or cystinuria, and analogous to structural abnormalities such as
polydactylism.

The origin of the sugar found in the urine in essential pentosuria
has been the subject of much debate and many experiments.   Its
most remarkable character is its optical inactivity, which marks
it out as a striking exception to the general rule that the animal
organism is built of optically active substance.   In one case
Neuberg succeeded in isolating the pentose from the di-phenyl-
osazone prepared from a large volume of the urine, and was able
to prove that it was racemic arabinose.   The optical inactivity of
the sugar proves that it is not derived from vegetable foodstuffs,
for the pentose they contain is dextro-rotatory l-arabinose; moreover,
the excretion of pentose continues when all pentosans are excluded
from the diet.   It is not likely to be derived from the pentose con-
tained in nucleo-proteins of the food, which is l-xylose, for Bial and
Blumenthal found no increases in the excretion of pentose after
feeding with 500 grams of calf's thymus.   Nor can it come from the
sugar of the nucleo-protein of the tissues, which is also l-xylose,
since, according to Gründ, the total amount in the human body is
about 10 grams, which would be less than one day's output in some
cases of pentosuria ; further, the uric acid and phosphate excretion
in cases of pentosuria gives no evidence of abnormal destruction
of nucleo-proteins.   On chemical grounds, too, the origin of r-ara-
binose from l-xylose is not probable.   The carbohydrate content
of the diet appears to bear no relation to the amount of pentose
appearing in the urine, and its total exclusion in no way influences
the output.   Dextrose and levulose are both made use of by
pentosurics as completely as by normal individuals, and even
pentoses, when given by the mouth, are destroyed in the same way
as they are by healthy persons.   Bial and Blumenthal found that
when 50 grams of l-arabinose were given to a patient with pentos-
uria only 6 grams reappeared in the urine, and Tintemann showed
that xylose behaved as with healthy persons, about 8 grams re-
appearing in the urine after 20 grams had been taken by the mouth.

There appears to be some evidence that the excretion of
r-arabinose is related to the proteins of the food and the activity
of the metabolic processes in the body.   Klercher found that the
output varied much during the day, and that there was a certain

parallelism between its excretion and the total nitrogen content of the urine. In one of his patients the lowest figure was obtained after fasting, and on that day there was an abnormally low nitrogen excretion. Klercher and Janeway also observed a diminished excretion on a purin-free and milk diet. Blumenthal and Meyer state that meat increases the nervous disturbance, and that milk is the most advantageous diet. The evidence that the excretion of the pentose is influenced by the proteins of the food is not, however, conclusive. Bial and Blumenthal found that the blood of a patient with pentosuria gave the orcin reaction, and concluded that a pentose was present, thus tending to exclude the renal origin of the sugar. Blumenthal states that pentoses were absent from the hydrocele fluid of one of his patients. Injection of phlorhidzin gives rise only to dextrosuria in pentosurics as in normal individuals, while the administration of chloral and menthol, although it causes an increased output of glucuronic acid in the usual way, does not influence the excretion of pentose. It may be pointed out that the differentiation of glucuronic acid from pentoses must be carefully made, for their chemical reactions are in many respects so closely alike that the one may be easily mistaken for the other ; in fact, it is not improbable that in some cases which have been described as examples of pentosuria (Caporelli, Colombini) the reducing substance was really glucuronic acid. Taking all the known facts into consideration, it would seem most probable that the sugar found in the urine in cases of essential pentosuria is derived from some substance formed within the organism, and that this parent substance is not dextrose. Neuberg has suggested that the most likely mother-substance is d-galactose. Theoretically such a conversion is possible, and it is known that galactose can be formed within the body, for it has been shown by Theirfelder to be the sugar yielded by cerebrin, and, with dextrose, forms the lactose of milk. At present there is no conclusive evidence of the correctness of Neuberg's hypothesis. Tintemann observed a slight increase in the amount of pentose in the urine after giving 50 grams of galactose on an empty stomach. Klercher noticed an increase in the hourly output for six or seven hours after 100 grams of lactose, but the total excretion was not excessive. Blumenthal and Bial found no conspicuous increase in the urinary pentose after 100 grams of galactose by the mouth. But further observation and experiment on this point is necessary.

An optically active arabinose is stated to have been found in the urine in three cases of chronic pentosuria, either alone, or with the inactive sugar. In Luzatto's case l-arabinose was believed to be

present, since the urine was dextro-rotatory and yielding an osazone with characteristic activities and melting-point, but the evidence is not very conclusive (Magnus-Levy). Blumenthal and Bial met with l-arabinose and the inactive variety together in one case, but here again the proof is doubtful, and the possibility of an alimentary origin for the optically active sugar was not excluded. Basing his conclusion on the spectroscopical characters of the orcin test, Brat concluded that a methyl-pentose (rhamnose) was present in his case of pentosuria.

Ribose, a reduction product of the lactone of ribonic acid which the investigations of Levene and Jacobs have shown to be contained in some nucleic acids, was stated by Elliott and Raper to be the pentose present in the urine of a case they examined.

Prognosis.—Essential pentosuria has been recognised for such a comparatively short time, and in such a small number of cases, that it cannot be definitely stated whether it does, or does not, ultimately shorten life, but it would appear that the presence of a sugar with five carbon atoms in its molecule in the urine has not the serious significance that attaches to the presence of dextrose. The condition may apparently persist unchanged for years without there being any increased liability to infection, or the occurrence of secondary disturbances of metabolism, such as result from persistent glucosuria. Blumenthal has suggested that the excess of circulating sugar may give rise to arterio-sclerosis, but there is no evidence in support of this. Some pentosurics have been members of diabetic families, small quantities of dextrose have been met with in the urine of a few cases, and in some cases of diabetes a pentose may be found, but all the available evidence is against the view that essential pentosuria increases the liability to chronic glucosuria. The prognosis therefore is good.

Treatment.—No form of treatment has been found to materially influence the condition, if we except Colombini's doubtful case. An anti-diabetic diet is unnecessary, and may do more harm than good, for not only is it irksome to the patient, but the indications are that a limitation of the proteins, rather than of the carbohydrates, is desirable. Morphine, and similar drugs, are contra-indicated, since chronic pentosuria has been associated with a drug habit in at least three cases and the administration of morphia has been found to increase the tendency to alimentary pentosuria. The most important reason why essential pentosuria should be watched for, and recognised, is that it needs no treatment ; the patient can thus be saved from the inconvenience, mental worry, and possibly financial loss that an incorrect diagnosis of diabetes mellitus would entail.

3. *Mixed Pentosuria and Dextrosuria.*—A certain number of patients with undoubted essential pentosuria have excreted dextrose in their urine along with the pentose. The original patient of Salkowski and Jastrowitz passed a small quantity of glucose, but, as it disappeared when the morphia he took was stopped, it is possible that the morphia habit may have been the exciting cause of the temporary glucosuria. One of Blumenthal's patients was glucosuric, and so also was one of Klercher's. The amount of dextrose in these cases was small (1·5 per cent. to 1·0 per cent.). There is, however, another class in which a large amount of glucose is associated with a small quantity of a pentose. The frequency of this association is variously estimated by different authors. Külz and Vogel investigated eighty diabetic urines and only failed to obtain indications of the presence of a pentose in four. With twelve a feeble, or doubtful, phloroglucin reaction was obtained, but with sixty-four the colour reaction and spectroscopic appearances were distinct and characteristic. Such evidence by itself would be of little value, but from several cases of severe diabetes they succeeded in isolating an osazone that was soluble in hot water and had the melting-point and nitrogen content of a pentosazone. As only small yields of this product, at most 0·1 gram per litre of urine, were secured the exact nature of the pentose was not determined. Bendix and other observers have failed to detect a pentose in the diabetic urines they examined, although they used the same method as Külz and Vogel, so that it would appear that the presence of a 5-carbon atom sugar in diabetes is not as common as the experience of the latter would suggest. Külz and Vogel also found a pentose in the urine of dogs rendered diabetic by removal of the pancreas, and it was noticed that, as in essential pentosuria in human beings, the excretion of pentose in the urine by these animals was not dependent upon the diet. The exact nature of the pentose was not determined. In only one recorded case, that of d'Amato, would it appear that the presence of a pentose along with dextrose in the urine was definitely associated with disease of the pancreas, but it is not unlikely that the pentose was of alimentary origin. My own observations on the urine with the modified pancreatic reaction, would suggest that in some 75 per cent. of cases of diabetes a non-fermentable reducing substance, probably a pentose in some, but in others possibly glucuronic acid, is present in the urine after it has been heated with hydrochloric acid.

Glucuronic Acid.—A small amount of glucuronic acid is a normal

constituent of the urine. Mayer and Neuberg state that it is present to the extent of 0·004 grams per 100 c.c., and Tollens and Stern found an average daily output of 0·35 to 0·37 grams. It does not occur in the free state, however, but as conjugate glucuronates, and it is to the presence of these that the feeble levo-rotatory power and, in part, the slight reducing effects of normal urine are due (Lavesson). The phloroglucin and orcin reactions given by normal urines, after they have been boiled for a minute or so with 1 per cent. sulphuric acid, are also referable to the presence of compound glucuronates.

Physiologically glucuronic acid appears to be part of the protective mechanism of the body by which the organism defends itself against harmful substances, formed in the tissues or introduced from without. Poisons of various kinds are usually rendered innocuous in one or more of four ways—(1) by being rapidly eliminated, (2) by being deposited and fixed in various organs or tissues, notably in the liver, (3) by being chemically altered through oxidation, reduction, hydrolysis, or neutralisation, (4) by being combined with substances formed, or contained, in the tissues, so that compounds of a harmless, or less toxic character than the original poison, result. The chemical defences employed against inorganic poisons are mainly the simple processes of oxidation, reduction, &c., but with the more complex organic poisons protective combinations are in addition very frequently formed. The chief protective substances employed for this purpose are alkalies, proteins, hydrogen sulphide, glycocoll, urea, bile salts, acetic acid, sulphuric acid, and glucuronic acid. Although most of these have special affinities for various chemical substances, depending on their composition, their action is not strictly specific, like the immune substances against bacteria and their product, and some are capable of replacing others, so the poison may be excreted partly in combination with one, and partly with another. Moreover, since most at least of the protective substances are not special bodies formed for the purpose of dealing with a poison appearing in the circulation, but are normal products of metabolism diverted to this end, when the available amount of any particular one is exhausted the residue of the poison must either unite with a substitute or go uncombined. We have already considered an example of this protective mechanism and seen how one neutralising substance is replaced, and augmented, by another, when dealing with the question of diabetic acidosis. In this case the acids formed as a result of the abnormal metabolic changes that are met with in severe cases of diabetes are first neutralised by the fixed

alkalies, but later, when there is a danger of the alkalinity of the blood being seriously reduced, they are combined with ammonia, derived from the nitrogen that normally goes to form urea.

Two of the most important protective substances are sulphuric and glucuronic acids, both of which have the power of combining with a number of toxic agents to form harmless, or comparatively harmless, compounds that are readily soluble and can be easily eliminated in the urine. They are most commonly met with in combination with various aromatic bodies formed in the intestine as a result of the cleavage of proteins. According to Herter, these are capable of setting up marked derangements of function, and probably even histological changes, when brought in contact with the elements of the nervous system in an unchanged condition, and the experiments of Woolley and Newburgh suggest that some of them, at least, may induce hyperactivity of the chromaffin tissue of the body with resulting pathological changes. As far back as 1877, it was shown by Baumann and Herter that when one of these substances, phenol, is given to animals it is excreted in the urine as a potassium salt of the sulphuric acid derivative, and later Magnus-Levy found that in carbolic acid poisoning, while some of the phenol appears in the urine in combination with sulphuric acid, a great part is eliminated as a glucuronic acid compound. It has also been shown that indol and skatol are similarly rendered innocuous by being converted into sulphuric and glucuronic acid compounds, but in this case after a preliminary oxidation into indoxyl and skatoxyl. It would thus seem that glucuronic acid and sulphuric acid have similar functions. Many observers have held that sulphuric acid is the first line of defence, and that it is only when there is not sufficient of this to combine with all the poison that the excess is excreted in combination with glucuronic acid. Salkowski has pointed out, however, that the latter may begin to be formed before the sulphuric acid is exhausted, and Tollens has shown that the lower derivatives of protein decomposition do not unite indifferently with sulphuric and glucuronic acid, but that indol given by the mouth is excreted mainly in combination with sulphuric, and phenol with glucuronic, acid. In health some tenth part of the total sulphuric acid of the urine is in combination with aromatic substances, as ethereal sulphates, and according to Tollens the excretion of glucuronic acid is, as a rule, about double this 0·35 grams of glucuronic to about 0·18 grams of ethereal sulphates. The former is chiefly in combination with phenol, but indoxyl and skatoxyl glucuronates are also present in smaller amounts.

The most striking demonstration of the way in which glucuronic acid is used by the organism to protect itself from the action of deleterious substances, is furnished by the abundant excretion of compound glucuronates that follows the administration of certain drugs, and other substances that contain an hydroxyl group, and are only oxidised with difficulty in the tissues. These include chloral, camphor, bromol, naphthol, aniline, benzine, turpentine, phenol, salicylates, borneol, resorcinol, menthol, toluol, thymol, antipyrin, antifebrin, and numerous other alcohols and ketones. It was formerly thought that the compounds formed under these circumstances were alcoholates, but they are now considered to be of a glucosidal nature. Their relation to the glucosides is shown by the action of appropriate glucoside-splitting ferments on them; thus phenol-glucuronic acid is attacked and slowly broken down by emulsin into phenol and glucuronic acid, while other compound glucuronates are gradually decomposed by invertin. Like the glucosides the conjugate glucuronic acids are hydrolised by mineral acids, yielding glucuronic acid and the particular alcohol from which they are derived, but some more readily than others. All conjugate glucuronic acids, however, do not exhibit the characters of glucosidal compounds, for some, such as urochloralic acid (tri-chlorethyl glucuronic acid) and paramidophenyl-glucuronic acid, for example, reduce alkaline solutions of copper as readily as dextrose, a reaction which is only obtained with most compound glucuronates after the acid has been set free by hydrolysis. This property appears to be due to the existence of a free aldehyde group in the compound. In consequence of the reducing powers possessed by some urines containing compound glucuronates, either immediately, or after prolonged boiling, it was formerly thought that the administration of certain drugs gives rise to temporary glycosuria, and, although this appears to be true of a few, the reduction obtained in most instances is dependent upon glucuronic acid.

The compound glucuronates formed naturally with the lower derivatives of protein decomposition are, like most of those re-sulting from the administration of drugs, of an ethereal or glucosidal nature, but show marked differences in the readiness with which they are split up. Indol-glucuronic acid, for instance, reduces alkaline solutions of copper on boiling for some time, but phenol-glucuronic acid is not readily decomposed and therefore does not reduce even after prolonged heating. The presence of an excess of the former in the urine may, therefore, give rise to a false idea that traces of sugar are present, unless care is taken and the results

of the reduction tests are confirmed in other ways. With the
latter such a mistake is not likely to arise. It will be remembered,
however, that indol is mainly excreted in combination with sul-
phuric acid, and it is probably only when more is being formed
than can be bound by the available sulphuric acid that a sufficient
quantity to give rise to difficulty is likely to be excreted in the form
of a glucuronate.

Pathological Excretion.—Very little is known concerning the
excretion of glucuronic acid in disease. An increased output has
been observed in various pathological conditions, but reliable
observations are not numerous, and the significance of an increased
excretion is not yet agreed upon in all cases. A number of sub-
stances derived from the aromatic radicals of the protein molecule
have been found in the intestinal contents, and some of these, as
we have seen, make their appearance in the urine in combination
with glucuronic acid even in health. Thus phenol, which is probably
derived from tyrosin, and the closely related paracresol, para-
oxyphenyl-acetic acid, and para-oxyphenyl-propionic acid are
apparently excreted chiefly as conjugate glucuronates. Indol-
propionic acid, indol-acetic acid, and the better known skatol and
indol are derivatives of tryptophan and are partly got rid of as
glucuronates, although mainly as ethereal sulphates. When for
any reason the putrefactive changes in the intestine are increased,
and these substances are consequently formed in excess, the output
of conjugate glucuronates is correspondingly augmented, while at
the same time the proportion of ethereal sulphates is increased.
The amount of sulphuric acid appearing in the urine in such organic
combination has long been considered as an index of the amount
of intestinal putrefaction, but very little attention has been paid
to the increase in conjugate glucuronic acid. According to Tollens,
the ethereal sulphates and the compound glucuronates rise and fall
together usually, but not always, proportionally to the amount of
intestinal putrefaction. In peritonitis, enteritis, and other con-
ditions promoting abnormal putrefactive changes in the intestinal
contents, there is also a marked increase in both. An increased
excretion of glucuronic acid has also been observed in association
with putrefactive changes in other situations—for example, with
gangrene, sloughing cancer, putrid placenta, or decomposing
exudates.

Although it does not appear probable that indol can be derived
from tryptophan liberated during intracellular protein metabolism,
it is not unlikely that para-oxyphenyl-acetic acid, para-oxyphenyl-
propionic acid, and other derivatives of tyrosin, &c., may be

formed in this way, and that this may account for the compound glucuronates found in the urines of patients with febrile diseases, respiratory difficulties, and impaired metabolism. I have met with an abnormal output of glucuronic acid in scarlet fever, smallpox, and measles, also in pneumonia, chronic bronchitis, and emphysema. In the course of a series of investigations with the phenylhydrazin test that I carried out some years ago, I found that out of a hundred urines from apparently healthy people, six gave a reaction with phenylhydrazin alone, and thirteen after boiling the urine with hydrochloric acid. On dividing the series into a set of forty-three derived from persons living in the country with an abundance of fresh air and exercise, and a second set of fifty-seven obtained from city dwellers who live under less favourable hygienic conditions, it proved that none of the former gave a reaction until the urine had been boiled with the acid, but that six of the second series gave a positive result before treatment, and nine after. Further investigation showed that in every case the reaction was due to glucuronic acid.

Early in my investigations into the condition of the urine in diseases of the pancreas I was struck by the marked increase in the excretion of glucuronic acid that accompanied inflammatory affections of the gland, and my subsequent experience has shown that it is a very constant phenomenon. At first sight one might be inclined to ascribe this to an excessive absorption of the products of putrefaction from the intestine, owing to the pancreatic disease interfering with the normal digestion of proteins, but the output of glucuronic acid does not appear to bear any relation to the amount of ethereal sulphates, or indican, in the urine, and is generally greater in the early stages of catarrhal pancreatitis than it is when there is advanced cirrhosis or malignant disease, where protein digestion is more seriously interfered with.

Many diabetic urines contain compound glucuronates, as much as 13·6 grams of the anhydride having been found by Baumgarten in one case. On administering drugs such as chloral, camphor, naphthol, &c., to patients with severe diabetes, as well as to dogs after extirpation of the pancreas, Weintraud found that these substances are eliminated in combination with glucuronic acid almost as abundantly as by normal individuals. In cases of chronic glucosuria where the elimination of sugar has ceased owing to careful dieting, glucuronic acid compounds are still often present in the urine in abnormal quantities.

Origin.—Chemically glucuronic acid is closely related to glucose,

two atoms of hydrogen in the CH_2OH group of the latter being replaced by one of oxygen in the former :—

$$CH_2OH - (CH.OH)_4 - COH \qquad COOH - (CH.OH)_4 - COH$$
$$\text{Dextrose.} \qquad\qquad\qquad \text{Glucuronic acid.}$$
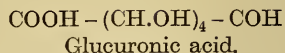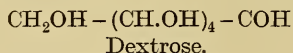
Since this process of oxidation involves no disturbance in the linkage of the carbon atoms in the sugar molecule, it would seem likely that it can be readily effected, and that the glucuronic acid that is excreted in the urine in the shape of compound glucuronates represents an early oxidation product of sugar, that is diverted from its further natural degradation to combine with toxic substances for the protection of the organism. The experiments of Jolles appear to support this view, for he showed that glucuronic acid is one of the products of the oxidation of sugar in weakly alkaline solutions.

Another hypothesis advanced by Sundvik, and later by Fischer and Pilot, has, however, been very generally accepted. According to this, the toxic substance combines directly with dextrose to form an ether-like compound. One end of the chain is thus shielded from attack by the pairing substance, but the other is open to chemical change. Subsequently it undergoes oxidation, and glucuronic acid results, the paired, or conjugate, glucuronates formed being then excreted in the urine. The faculty of removing injurious substances by combining them with glucose to form indifferent compounds is a striking feature of plant physiology, but whereas the glucosides of plants undergo no further change, and exist as such in the plant cells (e.g. amygdalin in almonds, salicin in willows, sinigrin in the cruciferæ, phloridzin in cherry trees, &c.), the more active oxidising tissues of the animal appear to change the glucose radicle into glucuronic acid :—

$$R.O.CH(CH.OH)_2.CH.CH(OH)CH_2(OH) \qquad R.O.CH(CH.OH)_2.CH.CH(OH)COOH$$
$$\text{Glucoside.} \qquad\qquad\qquad\qquad \text{Paired glucuronic acid.}$$
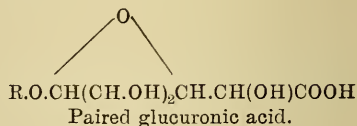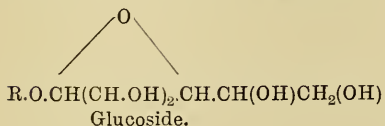
Whichever way glucuronic acid is formed, there can be little doubt that the parent substance is dextrose, and the question then arises, whence is the dextrose derived ? The first source that suggests itself is the carbohydrates of the food. Mayer has shown, however, that the administration of chloral, camphor, &c., to starving animals causes as great, or nearly as great, an excretion of compound glucuronates as when corresponding doses are given to well-fed animals. Hildebrand also found that bases of the type

of thymotinpiperidin, which are excreted in conjugation with glucuronic acid, are very little less poisonous when dextrose, cane-sugar, or maltose has been given beforehand than when they are taken by unprepared animals. It would seem, therefore, that glucuronic acid is not derived from the carbohydrate of the food, but from the glycogen contained in the liver and other reservoirs, or from the carbohydrate moiety of the proteins. In favour of the former origin is the observation of Embden that the passage of blood containing phenol through the liver of a dog, gives rise to the formation of phenol-glucuronic acid, and the fact that the administration of glucuronic acid with the food has been found to be followed by a deposition of glycogen. As the results of their experiments, Mendel and Jackson came to the conclusion that glucuronic acid is formed solely in the intermediary metabolism of proteins. These investigators gave camphor to fasting dogs for several days, and noted the output of glucuronic acid. They then gave large doses of dextrose, and found that protein metabolism fell, and with it the excretion of glucuronic acid. On adding meat to the diet a rise in the excretion of campho-glucuronic acid, corresponding to the amount of protein food ingested, occurred.

Most observers are agreed that the appearance of compound glucuronates in the urine is generally an expression of the power of the organism to deal with toxic substances, but, while many consider that this is the invariable explanation, there are others who maintain that it does not hold good in every case, and that in some instances an increased excretion may result from a perversion of the internal metabolism of the body. Mayer has put forward the view that the oxidative capacity of the body for dextrose may, under certain circumstances, be so far diminished that in part the process stops short at the formation of glucuronic acid, and occasionally may be insufficient to carry a portion even to this stage. The larger proportion of the circulating glucuronic acid then present combines with protein derivatives which would otherwise unite with sulphuric acid, and, as a result, there is a diminished excretion of conjugate sulphates. He accounts in this way for the increased output of compound glucuronates met with in febrile diseases and in conditions associated with respiratory difficulties, and also the occasional appearance of sugar in the urine in such cases. The excretion of sugar in febrile diseases is, however, a rare phenomenon, and it seems probable that implication of the pancreas, rather than a primary defect in oxidation, is responsible for the alimentary and spontaneous glycosuria that is occasionally met with. The glycosuria associated with respiratory difficulties

is of the asphyxial type, and this, as we have seen, is usually ascribed to stimulation of the nerve centres by the carbon dioxide present in the intensely venous blood. According to the protective mechanism view, the increased excretion of glucuronates in these cases is to be explained by supposing that the abnormal intermediate products of metabolism that are formed as a result of the defective tissue changes, unite with dextrose in the ordinary way, and subsequently undergo oxidation. The chief experimental evidence brought forward by Mayer in support of his hypothesis is furnished by observations on patients with diabetes and alimentary glycosuria. He was able to detect glucuronic acid in the urines of eleven out of thirty cases of diabetes, and found that when 100 to 200 grams of glucose were given to persons with alimentary glycosuria, fourteen excreted conjugated glucuronic acid along with the sugar, and six compound glucuronates alone. Blumenthal points out that it is difficult to understand how small amounts of dextrose and of glucuronic acid can be excreted together solely because the oxidising power of the organism for these substances is diminished, and thinks that it is probable that, since the conjugated acid is never found in the urine, even after it has been hypodermically injected, it is most likely that in such cases the formation of the substance with which it combines precedes the formation of the acid. The observations of Achard and Weil, and of Strauss, tend to confirm Blumenthal's explanation, for the former found indoxyluria in a case of alimentary glycosuria that gave similar results, which they investigated, and the latter points out that indoxyluria is very common in diabetes, and that the increased excretion of glucuronic acid stands in very close relation to it.

Mayer explains the occurrence of oxaluria in diabetes mellitus on the assumption that more glucuronic acid is formed than can combine with the available quantities of protein decomposition products, and that this is oxidised to oxalic acid, which combines with calcium, and is so excreted. He points out that oxaluria is apt to follow the ingestion of large amounts of glucose in diabetes, and that when a diabetic has so far recovered his powers of metabolising carbohydrates that the sugar in the urine diminishes, it may be partly replaced for a time by oxalates. Mayer gave 10 grams of sodium glucuronate to rabbits, and found that its administration was followed by the appearance of saccharic acid and a large amount of oxalic acid in the urine. He also found oxalic acid in the liver, and states that oxalic acid is a product of the autolysis of glucuronic acid in the liver. In this connection it is interesting to note that a deposit of calcium oxalate crystals,

and an increased excretion of oxalic acid, is in my experience a much too frequent occurrence to be accidental in chronic pancreatitis. It is met with most frequently in old standing cases where there is considerable cirrhosis of the gland, but not yet sufficient to give rise to glycosuria.

A point of considerable interest in connection with the chemistry of glucuronic acid must be mentioned here, and that is its relation to the pentoses. Ruff has shown that by the action of certain oxidising agents, and particularly hydrogen peroxide in the presence of ferric acetate, d-arabinose can be obtained from the potassium salt of d-glucuronic acid, and Salkowski and Neuberg have demonstrated that l-xylose can be derived from glucuronic acid by the action of putrefactive bacteria. The latter observation is particularly important, for l-xylose is a very constant constituent of the cell nuclei of the body, more especially of the pancreas, and if this change can be carried out by bacteria it is not unlikely that it can also be effected by animal ferments in a similar way. It is worthy of note that an increased excretion of glucuronic acid is very constantly associated with inflammatory affections of the pancreas, and that a substance which on hydrolysis yields a body giving the reactions of a pentose may, according to my experience, be also obtained.

Recognition.—The presence of glucuronic acid in the urine, or other fluids of the body, is most satisfactorily demonstrated by decomposing the conjugate glucuronates with 1 per cent. sulphuric acid in the autoclave, and preparing the p-brom-phenylhydrazin compound. This is characterised by its high melting-point, 236° C. (200° to 216° C. in the impure form), its insolubility in absolute alcohol, and its high degree of levo-rotation in pyridin-alcohol solution ($-7° 25'$). Clinically, glucuronic acid compounds may be recognised in the urine by the negative result of the fermentation test ; by the urine being levo-rotatory, even after fermentation ; the change produced in the rotatory power by boiling with acids, the levo-rotation being diminished, or replaced by a dextro-rotation ; by an increase in the reducing power of the urine after it has been boiled with dilute sulphuric acid and neutralised ; also by the fact that the orcin test which was previously negative, or only given after prolonged boiling, is at once positive after treating the urine with dilute sulphuric acid. Tollens's naphthoresorcinol test may also be used, but as it gives a positive reaction with many normal urines, owing to the conjugate glucuronic acid they contain, the result must be well marked before it can be concluded that an abnormal amount is present.

BIBLIOGRAPHY

LACTOSE

Blumenthal, *Path. d. Harnes*, 1903.
Borchardat and Finkelstein, *Deut. med. Woch.*, 1893.
Bourquelot and Troisier, *Compt. rend. d. Soc. d. Biol.*, 1889.
Gérard, *Ann. d. Gynécol.*, xxxvii.
Grósz, *Jahrbuch. f. Kinderheilk*, 1892.
Halász, *Orvosi Hetilap*, 1906 ; *Deut. med. Woch.*, 1908.
Hess, Naunyn's *Diab. Mellit.*, 1907.
Hofmeister, *Zeit. f. phys. Chem.*, 1877.
Kaltenbach, *Zeit. f. phys. Chem.*, 1878.
Langstein and Steinitz, *Hofmeister's Beitr.*, 1906.
Lemaire, *Zeit. f. phys. Chem.*, 1895.
MacCann, *Lancet*, 1897.
Méhu, *Chem. Centralb.*, 1887.
Meyer, *Mundl. Mitteil.*
Naunyn, *Der Diab. Mellit.*, 1807.
Ney, *Arch. f. Gynäkol*, 1889.
Pavy, *Lancet*, 1897.
Porcher, *Bull. d. Soc. d. méd.*, 1902 ; *Biochem. Centralb.*, 1910.
Voit, *Zeit. f. Biol.*, 1892.
Worm-Müller, *Pflüger's Arch.*, 1884.
Zuelzer, *Centralb. f. med. Wissensch.*, 1894 ; v. Noorden's *Handb. d. Path. u. Stoffwech.*, 1907.

GALACTOSE

Bauer, *Wien. med. Woch.*, 1906.
Grósz, *Jahrb. f. Kinderheilk.*, 1892.
Langstein and Steinitz, *Hofmeister's Beitr.*, 1906.
Voit, *Zeit. f. Biol.*, 1892.

SACCHAROSE

Brown, *Johns Hopk. Hosp. Bull.*, 1900.
Hirschberg, *Lancet Clinic*, 1912.
Levy, v. Noorden's *Handb. d. Path. u. Stoffwech.*, 1906.
Reuss, *Wein. klin. Woch.*, xxiii.
Smolenski, *Zeit. f. phys. Chem.*, 1909.
Voit, *Deut. Arch. f. klin. Med.*, 1897.

PENTOSE

Adler, *Pflüger's Arch.*, 1905.
D'Amato, *Rev. crit. Klin.*, 1902.
Barczewski, *Gaz. Lekaraska*, 1897.
Bendix, *Münch. med. Woch.*, 1903 ; *Die Pentosurie*, 1903.
Bial, *Zeit. f. klin. Med.*, 1900 ; *Berl. klin. Woch.*, 1904 ; *Berl. Klinik*, 1907.

Bial and Blumenthal, *Deut. med. Woch.*, 1901.

Blum, *Zeit. f. klin. Med.*, 1906.

Blumenthal, *Berl. klin. Woch.*, 1895 ; *Path. d. Harnes*, 1903 ; v. Noorden's *Clin. Med.*, 1906.

Brat, *Zeit. f. klin. Med.*, 1902.

Cassiver and Bamberger, *Deut. med. Woch.*, 1907.

Chobola, *Centralb. f. inn. Med.*, 1907.

Colombini, *Monats. f. prakt. Dermatol.*, 1897.

Cominotti, *Biochem. Zeit.*, 1909.

Cremer, *Zeit. f. Biol.*, 1882, 1893.

Ebstein, *Virchow's Archiv.*, 1892–3.

Elliott and Raper, *Journ. Biol. Chem.*, 1912.

Funaro, *Arch. farmac. sperim.*, 1907.

Garrod, *Inborn Errors of Metabolism*, 1909.

Gründ, *Die Pentosurie*, 1903.

Von Jaksch, *Centralb. f. inn. Med.*, 1906.

Janeway, *Amer. Journ. of Med. Sci.*, 1906.

Johnstone, *Edin. Med. Journ.*, 1906.

Jolles, *Zeit. f. anal. Chem.*, 1907 ; *Münch. med. Woch.*, 1908.

Kaplan, *New York Med. Journ.*, 1906.

Klercher, *Nord. med. Arch.*, 1905.

Kraft, *Pharmaceut. Centralb.*, 1906.

Külz and Vogel, *Zeit. f. Biol.*, 1895.

Luzzato, *Arch. di Farmacol*, 1902 ; *Arch. f. exp. Path.*, 1908.

Meyer, *Berl. klin. Woch.*, 1901.

Neuberg, *Berich. d. deut. Chem. Gesellsch.*, 1900 ; *Ergebenisse. d. Physiol.*, 1904 ; v. Noorden's *Path. d. Stoffwech.*, 1907.

Reale, *Riv. Clin.*, 1894 ; *Centralb. f. inn. Med.*, 1894.

Romme, *Presse médicale*, 1901.

Rosenfeld, *Mediz. Klinik.*, 1906.

Salkowski, *Zeit. f. phys. Chem.*, xxvii. ; *Berl. klin. Woch.*, 1895.

Salkowski and Jastrowitz, *Centralb. med. Wissensch*, 1892.

Schüler, *Münch. med. Woch.*, 1905.

Thierfelder, *Zeit. f. phys. Chem.*, 1890.

Tintemann, *Zeit. f. klin. Med.*, 1905–6.

Vas, *Orvosi Hetilap*, 1907 ; *Wien. klin. Woch.*, 1908.

Wall, *Amer. Journ. Pharm.*, 1909.

GLUCURONIC ACID

Baumann and Herter, *Zeit. f. phys. Chem.*, 1877.

Baumgarten, *Zeit. f. exp. Path.*, 1906.

Blumenthal, *Arch. f. Phys.* (supl.), 1901.

Cammidge, *Proc. Roy. Soc.*, 1909.

Embden, *Hofmeister's Beitr. z. chem. Phys.*, 1901.

Fischer and Pilot, *Ber. d. deut. chem. Ges.*, 1891.

Herter, *New York Med. Journ.*, 1898.

Hildebrand, *Arch. f. exp. Path.*, 1900.

Jolles, *Wien. med. Woch.*, 1911.

Lavessen, *Biochem. Zeit.*, 1907

Levy, *Münch. med. Woch.*, 1905.

Mayer, *Deut. med. Woch.*, 1901 ; *Zeit. f. klin. Med.*, 1902.

Mayer and Neuberg, *Zeit. f. phys. Chem.*, 1900.

Mendel and Jackson, *Amer. Journ. Phys.*, 1902.

Ruff, *Ber. d. deut. Chem.*, 1898.

Salkowski, *Zeit. f. phys. Chem.*, 1904.

Salkowski and Neuberg, *Zeit. f. phys. Chem.*, 1902.

Strauss, *Deut. med. Woch.*, 1902.

Sundvik, *Jahresb. f. Tierchem.*, 1886.

Tollens, *Zeit. f. phys. Chem.*, 1910.

Tollens and Stern, *Zeit. f. Phys. Chem.*, 1910.

Weintraud, Naunyn's *Diab. Mellit.*, 1907.

Woolley and Newburgh, *Journ. Amer. Med. Assoc.*, 1911.

Alkaptonuria.—In 1858 Bödeker detected in the urine of a patient with glycosuria a second reducing substance to which, on account of its behaviour with alkalies, he gave the name alkapton. This substance, in spite of its reducing powers, was found not to be a sugar, but to contain nitrogen. Other observers who investigated subsequent cases of alkaptonuria held different views as to the nature of " alkapton "; some came to the conclusion that it was pyrocatechin, others thought it was protocatechutic acid, Marshall named it glycosuric acid, and Kirk, who separated an acid from the urines of a group of cases that he investigated, called it uroleucic acid. In 1891 Wolkow and Baumann isolated and fully investigated homogentisic acid, the excretion of which is undoubtedly the essential feature of alkaptonuria. The work of these, and other investigators, has definitely proved that this substance has the empirical formula $C_8H_8O_4$, and that it has the constitution of para-di-oxybenzene-acetic acid (hydroquinone-acetic acid).

Alkaptonuria is a very rare condition, and, although of great interest to the chemical physiologist and pathologist, would not be clinically important were it not that it may be mistaken for a trouble of a much graver kind if the fact of its existence, and the methods of differentiating it, are not borne in mind. The copious reduction that occurs when an alkaptonuric urine is heated with Fehling's solution will, to the uninitiated, suggest the presence of sugar, but the dark brown colour of the liquid in which the copper precipitate is suspended gives it a peculiar appearance which, to the experienced eye, indicates the true cause of the reduction. Nylander's solution darkens on being heated with the urine, but no precipitate of reduced bismuth forms, as it does with the sugars. On adding a dilute solution of ferric chloride to the urine, drop by drop, a transient deep blue coloration is seen to follow each addition, and is characteristic of homogentisic acid. The urine does not ferment with yeast, is optically inactive, and does not form an osazone with phenylhydrazin. When freshly passed the

urine of an alkaptonuric person seldom exhibits any abnormality of tint, but on exposure to the air it quickly darkens, especially if it is made alkaline and is gently warmed. In some instances attention has been drawn to the existence of the condition by linen, and other fabrics, soiled with the urine blackening on exposure to the air. Crystals of uric acid deposited from the urine are found to be stained brown. Beyond the presence of homogentisic acid the urine of alkaptonurics shows no striking or constant variation from the normal.

In the great majority of instances alkaptonuria is present from birth, and persists throughout life. It may attract attention shortly after the child is born through the staining of the clothes, or it may pass unnoticed until adult life is reached, when its presence is discovered in the course of an examination of the urine for life insurance or some other purpose. A few cases have been recorded in which it has appeared as a temporary condition, but according to Garrod the evidence of its temporary nature is doubtful in some, and in others the fact that the urine contained homogentisic acid was not completely established. In only one of them was a quantitative estimation carried out. This patient, who was under the care of Zimnicki, had intermittent alkaptonuria and suffered from hypertrophic cirrhosis of the liver. Geyger's patient was a diabetic and also had intermittent alkaptonuria. Hirsch described the case of a girl of seventeen, with febrile gastro-enteric catarrh, who passed for three days only a urine which darkened on standing, contained indican, and also yielded the alkapton reactions. A somewhat similar case came under my notice in 1903. The patient was a woman, aged thirty-two, in the City Fever Hospital, Newcastle-on-Tyne, under the care of Dr. S. G. Mostyn, who sent me the urine. This was of a dark colour, specific gravity 1·012, and contained a trace of albumen. It reduced Fehling's solution, but not Nylander's solution, and gave no osazone with phenylhydrazin. A dilute solution of ferric chloride produced a transient blue coloration and darkened the fluid. A fairly well-marked reaction for indican was also obtained. As the quantity of urine was too small for a detailed investigation, further proof of the nature of the condition was not possible, and a second specimen sent to me for analysis did not give the alkapton reactions. The darkening of the urine on standing was only observed for three days in all. The patient was lost sight of when she left the hospital, so that I cannot say whether the condition recurred or not.

Homogentisic acid is apt to be found in the urines of several brothers and sisters of a family whose parents do not exhibit the

anomaly, and Garrod has pointed out that a striking proportion of
alkaptonurics are the offspring of the marriage of cousins.   Ac-
cording to Bateson and Punnett, the mode of incidence of alkapton-
uria finds a ready explanation if it be regarded as a rare recessive
character in the Mendelian sense.   Like chronic pentosuria, alkap-
tonuria is now considered to be an inborn error of metabolism.
It is believed to be due to a failure of the organism to deal with
the aromatic fractions of the proteins of the food and tissues in the
ordinary way, and not to an abnormal formation of homogentisic
acid within the body, or to its production in the intestine from
putrefactive changes, as was at one time thought.   It appears to
depend upon a lack of ability of the alkaptonuric individual to
split open the benzene ring of the tyrosin and phenyl-alanin formed
in the intermediary metabolism of proteins.   Normally these
first suffer a splitting-off of the nitrogen radical from the alanin
side chain, followed by oxidation to homogentisic acid.—

Tyrosin.   Phenyl-alanin.   Homogentisic acid.
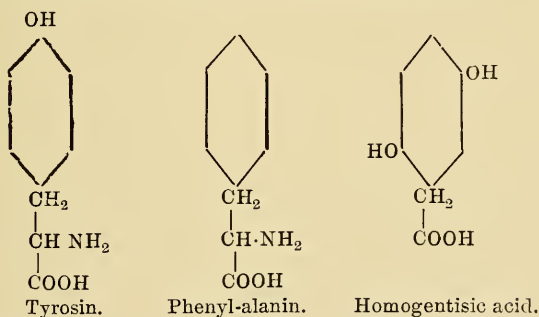
Then comes a disintegration of the benzene ring with subsequent
complete oxidation.   Alkaptonurics can carry out the conversion
as far as the oxy-acid stage, but there the process stops, and the
acid is excreted unchanged in the urine.   The relation of homo-
gentisic acid to the aromatic radicals of the proteins has now been
definitely established by an experiment carried out by Abderhalden,
who succeeded in inducing alkaptonuria in a healthy man who had
never previously exhibited the condition by feeding him with
50 grams of tyrosin a day, a quantity corresponding to many
hundred grams of protein.   This experiment does not throw any
light on the nature of the perversion, but it is possible that the
catabolism of the aromatic fractions of the proteins is carried out
by a series of special enzymes, and it has been suggested that
alkaptonuria arises from the absence of the ferment which normally
has the power of splitting the benzene ring.

Alkaptonuria gives rise to no symptoms, with the exception

of occasional dysuria and undue frequency of micturition. In a few instances that peculiar staining of the tissues to which Virchow gave the name of ochronosis, has developed in later life.

Diabetes Insipidus.—Diabetes insipidus is a condition characterised by the persistent passage of an excessive quantity of urine of low specific gravity, and without any constant abnormal constituent. It is probably not a disease, but a symptom that results from several morbid conditions, and will be briefly considered here because the clinical manifestations of diabetes insipidus resemble those of diabetes mellitus in some respects, and there is experimental evidence that the two conditions may be set up by similar lesions.

The polyuria associated with hysteria, nervous excitement, high tension, arteriosclerosis, hydronephrosis, and chronic interstitial nephritis should be distinguished from diabetes insipidus, for, although the amount of urine excreted is increased in all of them, it is represented by pints rather than by quarts, and the cause of the condition is obvious.

Etiology.—True diabetes insipidus is a rare condition. It may exist in the new-born infant, or appear in old age, but is most commonly met with in early adult life. It is about twice as frequent in males as in females. Heredity appears to play some part in its etiology, a history of polyuria, glycosuria, or albuminuria in previous generations being not uncommon. Gee has reported an example of the transmission of diabetes insipidus through four generations. A history of tuberculosis in the family is, according to Haussen and Bertrand Dawson, too frequent to be a mere coincidence. The onset of diabetes insipidus appears to be connected in some cases with nervous affections, nervous excitement, acromegaly, syphilis, blows or injuries of the head, or of the trunk or limbs. Sudden exposure to cold, alcoholic excess, convalescence from acute febrile diseases, malnutrition, and occasionally the presence of abdominal or thoracic tumours, have been stated to be the exciting cause.

Symptoms.—As a rule diabetes insipidus comes on slowly and insidiously, but the onset is sometimes sudden, especially after a fright or injury. Unquenchable thirst and polyuria are the outstanding features of the condition. The appetite is usually good, but is rarely excessive, as in diabetes mellitus. The bowels are often confined, and the patient sometimes complains of flatulence. The mouth is dry, the skin is harsh, and may become atrophic and withered, but boils and cutaneous lesions are rare. Itching of the

skin is sometimes complained of. The patient may be well nourished and healthy-looking, but the loss of sleep and distress consequent on the great thirst and frequent urination, sooner or later interfere with the general health and the patient becomes thin and debilitated, his temper is irritable, he may complain of distressing headache and a dull aching pain in the back. There is a loss of sexual power, the knee jerks which at first are often exaggerated may disappear, and there is a subnormal temperature. In some cases the blood pressure is normal, in others it is raised, but it is often abnormally low. Later the appetite fails, the emaciation becomes more marked, great weakness supervenes, the tongue becomes dry and glazed, attacks of diarrhœa may occur, and death takes place from exhaustion, a low form of pneumonia, or coma, if the patient has not been meanwhile carried off by some intercurrent affection, such as pulmonary tuberculosis, pneumonia, &c. As a rule the progress of the condition is slower, and the prognosis better, when it comes on without any obvious cause, than when it is associated with organic disease or injury. In the former type of case the general health of the patient may be well maintained for a lengthy period, and Osler states that the affection has been known to persist for fifty years. Spontaneous cure may occasionally take place, and has been known to follow an intercurrent disease, such as typhoid fever. A few cases have yielded to treatment, but as a rule the condition is intractable.

The amount of urine passed varies from 10 to 40 pints, or more, in the twenty-four hours, according to the quantity of fluid consumed, but it is usually much in excess of that met with in diabetes mellitus. It is clear, pale, of a greenish yellow colour, faintly acid in reaction, and of very low specific gravity, 1·001 to 1·006, but usually about 1·005. As a rule the total amount of solid constituents is about normal, although the percentage is of course low. Meyer states that the concentration of the urine tends to remain uniform, and that the amount of water is varied to regulate the concentration according to the amount of solid eliminated. If the nature of the diet is taken into account the output of urea is not excessive. Some observers have reported an increased excretion. Gerhardt, for example, met with 70 to 80 grams a day, but this was explained by the increased appetite and consequent consumption of a large amount of food by his patient. In other cases the urea excretion has been subnormal, and, as a daily output of less than about 20 grams a day renders the patient liable to become uremic, it is important that regular estimations of the total excretion of urea should be made in diabetes insipidus. Uric acid, creatinin,

sulphates, and phosphates are usually present in normal quantities, but occasionally an excess of phosphates has been found. Teissier met with 6·6 grams, and 37·5 grams of urea, in one of his cases, and suggests the name " Diabéte phosphatique " for this type of diabetes insipidus. The urinary constituent which is most commonly, although not constantly, increased is inosite, which is sometimes present in considerable quantities, 18 to 20 grams in the twenty-four hours. Its significance is not well understood, but Strauss has shown that it is probably related to the excessive consumption of water, and consequent polyuria. Acetone, aceto-acetic-acid, and the other products of abnormal metabolism met with in diabetes mellitus are not seen in cases of pure diabetes insipidus. Occasionally traces of albumen are found in the urine, and in some cases sugar is also present, especially if there is a lesion of the nervous system.

Pathology.—Diabetes insipidus, like diabetes mellitus, presents no constant anatomical lesions. Sometimes it has been found associated with tumours, syphilitic or tuberculous growths, or aneurisms, in the pons, medulla, or cerebellum. In others there has been fracture of the base of the skull, or mechanical injury to the brain. In one reported case an abdominal tumour, and in two a thoracic aneurism, was present. Many have shown no gross morbid change after death, excepting those due to intercurrent disease, and alterations in the urinary system, such as enlarged and congested kidneys, dilated pelves, dilated ureters, and an hypertrophied bladder, which might be ascribed to the passage of an abnormal amount of urine.

As the result of a study of the metabolism in diabetes insipidus, Tallqvist has suggested that the polyuria may be due to defective resorption of water by the renal tubules, and a number of authors, including Strubell, Meyer, and Seiler, have stated that there is a special functional disorder of the kidneys consisting of a loss of ability to concentrate the urine. If this disability exists it would necessitate a large volume of urine being excreted to remove the waste products from the body, and this in its turn would cause the consumption of an abnormally large quantity of liquid. If the requisite amount of water were not taken the tissue fluids would be drawn upon, or a retention of the solid urinary constituents would occur, with resulting uremia. The kidneys of certain patients do appear to have a difficulty in excreting concentrated solutions, and this has been found to apply more especially to solutions of sodium chloride, and, to a less extent, of urea. If such a person is given from 10 to 20 grams of sodium chloride with his food the

concentration of the salt in his urine is only slightly increased (from 0·1 to 0·2 per cent.), and a large amount of urine, and a considerable time, are therefore necessary for the excretion of the whole of the salt taken. This fact has been made use of by Minkowski to differentiate between polyuria due to inability of the kidneys to concentrate the urine, and an increase in the amount of urine excreted arising from other causes. According to Schmidt, the increased flow of urine in diabetes insipidus is dependent upon dilatation of the vessels of the kidneys without increased arterial pressure, and, since he found that a similar condition can be produced in animals by cutting the renal nerves, it is assumed that the vaso-dilatation is dependent upon nervous influences, due either to local irritation, as from abdominal tumours, &c., or to central causes. Bernard showed that experimental puncture of the floor of the fourth ventricle just above the diabetic centre, produces copious diuresis in animals, and it has also been found that injuries to the central lobe of the cerebellum, corresponding to the vermiform process of the human brain, will in certain animals produce a similar result, so that the causal relation of central lesions in, or about, these regions to diabetes insipidus receives confirmation on experimental grounds. These experiments also offer an explanation of the appearance of sugar in the urine in some cases. The observations of Goetsch, Cushing, and Jacobson on the effects produced by partial removal of the hypophysis cerebri in animals, also throw light on the connection between tumours, or injuries, of the brain and diabetes insipidus, for they show that hyperactivity of the hypophysis, such as would be caused by lesions stimulating the posterior lobe, is accompanied by polyuria, and, as we have already seen, is liable to give rise to glycosuria. Schäfer showed that in dogs constant mechanical irritation of the hypophysis causes permanent diabetes insipidus and a tendency to adipose-genital dystrophia. The conclusion seems inevitable that the internal secretion of the hypophysis controls the activity of the kidneys, and that essential diabetes insipidus may arise from excessive functioning of the gland. In support of this there is also considerable clinical evidence. Thus in Hagenbach's case, in which polyuria and polydipsia occurred in a little girl, cheesy tubercle of the infundibulum was found post-mortem. Rosenhaupt has described a case in which fever, thirst, and polyuria came on abruptly, and necropsy two weeks later revealed a sarcoma in the anterior lobe of the hypophysis. Frank reports a case in which disturbances similar to those produced in experimental research on the hypophysis was brought about by a bullet. The patient,

a man aged thirty-nine, became epileptic several years after he had tried to commit suicide by firing two bullets into his right temple. The balls could be seen in the head, one near to the cortex, and the other close to the sella turcica. The latter evidently kept up a constant mechanical irritation of the hypophysis and induced the polyuria, &c., from which he suffered. The brain affections which most usually accompany diabetes insipidus are those in which there has been traumatic concussion, and, as the region of the hypophysis is particularly liable to suffer in trauma of the skull, it is not unfair to assume that the resulting cicatrical changes exert a permanent irritating effect on the gland, either by direct pressure, or from interfering with the flow of cerebro-spinal fluid. Tumours of the brain may also act in a similar manner. A typical case of diabetes insipidus resulting from injury of the skull and followed by glycosuria probably brought about in this way has been described by French and Ticehurst :—

A man, aged forty-four, after a fall from his bicycle remained unconscious for fourteen days, with cerebro-spinal fluid dripping from his nose. On recovery, bilateral temporal hemianopia and ocular paresis showed that he had fractured the base of his skull near the chiasma. Previous to the accident he had been perfectly well ; after it he had extreme polyuria and thirst, passing up to 10,000 c. cm. of urine, specific gravity 1004·6, free from albumen and sugar. The expectation that the " sugar centre " in the medulla was injured led to extensive metabolism research in which all food and excreta were analysed. By increasing the carbohydrates in the food, it was attempted to produce glycosuria. Dextrose, starch, and cane-sugar were assimilated well, up to 700 grams (dry) of carbohydrate in twenty-four hours. No sugar could thus be made to appear in the urine. Two years later, however, glycosuria developed spontaneously. The urine remained of comparatively low specific gravity. Careful dieting did not entirely prevent glycosuria, and both acetone and diacetic acid were present as well as sugar. On ordinary diet there were no acetone and diacetic acid.

Diagnosis and Treatment.—If the condition can be assigned to any definite cause an attempt must be made to deal with it, but if none can be discovered, and the case is apparently one of " idiopathic " diabetes insipidus, it is necessary to determine (1) whether the increased excretion of urine is a primary disorder, which will lead to a reduction in the water-content of the organism unless large quantities of fluid are taken, or (2) whether the real cause of the polyuria is an excessive thirst accompanied by excessive drinking ? An answer to these questions can be obtained by observing the result of a reduction in the intake of water on the

amount of urine passed, being careful meanwhile to watch the effect on the composition of the urine, and particularly the daily excretion of urea. If there is a primary polydipsia the excretion of urine should be reduced by limiting the quantity of water taken, but if this result does not follow very quickly the inference is that the polyuria is the primary disorder. In the latter case there will also be a marked loss of weight, due to removal of water from the tissues. One may then proceed to attempt to differentiate between a polyuria dependent upon inability of the kidneys to concentrate the urine, and polyuria due to some other cause, by Minkowski's method. If after giving 10 to 20 grams of sodium chloride the urine of the next twenty-four hours contains practically all the salt, the condition is not primarily renal, and the patient may be safely placed on a limited amount of water. The limitation of the fluids must, however, be carried out with care and be guided by analyses of the urine, for if it is found that large quantities of urine are being passed, even with a limited intake of water, it is obvious that the patient must be extracting fluid from his own tissues with resulting immediate and perhaps permanent injury, and the supply must be increased. If, on the other hand, very little of the salt is excreted without increasing the water intake, the inference is that the excretory powers of the kidneys are at fault, and the best treatment would appear to be to place the patient on a diet poor in sodium chloride and nitrogenous foods. In one patient treated by this method Minkowski obtained a reduction in the volume of urine from 12 or 14 litres to 3 or 4 litres a day. Even if a restriction of the chlorides does not bring about such a remarkable result as this, it will tend to diminish thirst and so assist in the treatment of the case. A nitrogen-poor and salt-free diet cannot, however, be persisted in indefinitely, but by watching the weight and general metabolism it may be possible to gradually relax it as the condition of the kidneys improves until a more normal diet is reached, the sodium chloride being still adjusted as far as possible to the tolerance of the patient.

Patients suffering from diabetes insipidus should be kept free from worry and anxiety. Their clothes should be warm, and the food be made as nourishing and abundant as possible, with the limitations mentioned. Toxæmia should be carefully guarded against by regulating the bowels. The distressing thirst may be assuaged somewhat by acid drinks, or by sucking ice dipped in lemon-juice. Tea, coffee, and alcoholic beverages are to be avoided, although the tolerance for alcohol is in some cases remarkable, a couple of pints of brandy, or a dozen or more bottles of wine,

having been consumed in a day (Osler). A bracing climate, or a sea-voyage, is a useful adjuvant to the treatment in some cases. The most generally useful drugs are tonics, such as arsenic, iron, quinine, and strychnine. Anti-spasmodic remedies have been much used, and of these valerian was highly recommended by Trousseau, who gave it in enormous doses. Antipyrin, salicylate, and turpentine have been found useful in some cases, but they should be given with caution. Opium and its alkaloids are worse than useless in diabetes insipidus, for although the thirst and polyuria may be diminished by their use, they greatly increase the patient's discomfort, and in some instances have proved fatal. Occasionally they may be cautiously administered to combat sleeplessness and nervous symptoms, but, as a rule, bromides, unless specially contra-indicated, are to be preferred. Diuretics have not given favour-able results. If the blood pressure is low, vaso-constrictor drugs, especially ergot and extract of the pituitary gland, are indicated, since these raise the blood pressure and tend to prevent cerebral congestion and exudation. It is also possible that pituitary extract may have a specific action in some cases, but unfortunately the results are not permanent. In cases where the blood pressure is high, it should be lowered by appropriate baths, massage, physical exercise, change to a warm climate, diet, and vaso-dilator drugs, such as nitro-glycerine, &c. Finally, the effect of electrical treatment may be tried by applying the positive pole of a constant current of 1 to 4 milliamperes to the nape of the neck, and the negative pole, properly insulated, to the posterior naso-pharyngeal wall. Herrick has recently reported a remarkable improvement in a case of diabetes insipidus of four years' standing, possibly due to a lesion of the hypophysis, following the withdrawal of 5 c.c. of cerebro-spinal fluid by lumbar puncture. The urine, which before the operation had varied in amount from 7,500 to 11,000 c.c., with a specific gravity of 1·001, never exceeded 1,800 c.c., with a specific gravity averaging 1·015, during the succeeding four weeks that he was under observation. The thirst at the same time disappeared.

BIBLIOGRAPHY

ALKAPTONURIA

Abderhalden, *Lehrb. d. phys. Chem.*, 1906 ; *Zeit. f. phys. Chem.*, 1912.
Bateson, *Rep. Evol. Commt. Roy. Soc.*, 1902.
Bödeker, *Zeit. f. rat. Med.* 1859 ; *Ann. d. Chem. u. Pharm.*, 1861.
Falta, *Biochem. Centralb.*, 1904 ; *Deut. Arch. f. klin. Med.*, 1904.
Garrod, *Lancet*, 1902 ; *Inborn Errors of Metabolism*, 1909.

Geyger, *Pharmakeut. Zeit.*, 1892.
Hirsch, *Berl. klin. Woch.*, 1897.
Kirk, *Journ. Anat. and Phys.*, 1889 ; *Brit. Med. Journ.*, 1888.
Marshall, *Medical News*, 1887.
Punnett, *Proc. Roy. Soc.*, 1908.
Virchow, *Virchow's Arch.*, 1866.
Wolkow and Baumann, *Zeit. f. phys. Chem.*, 1891.
Zimmick, *Abst. Centralb. f. Stoffwech. u. Verdauungsk.*, 1900

DIABETES INSIPIDUS

Dawson, Allchin's *Man. of Med.*, ii., 1900.
Frank, *Berl. klin. Woch.*, 1912.
French and Ticehurst, *Brit. Med. Journ.*, 1906.
Gee, *St. Barthol.'s Hosp. Rep.*, 1877.
Gerhardt, *Der Diab. Insip.*, 1898 ; *Spec. Path. u. Therap.*, vii. 7, 1899.
Goetsch, Cushing, and Jacobson, *Johns Hopk. Hosp. Bull.*, 1911.
Haussen, *Norsk. Mag. f. Laegevidensk.*, 1912.
Herrick, *Arch. Internal. Med.*, 1912.
Külz, *Maly's Jahrb.*, 1875–6.
Meyer, *Deut. Arch. f. klin. Med.*, 1905.
Minkowski, *Die Therap. d. Gegenwart*, 1910.
Osler, *Princip. and Pract. of Med.*
Schmidt, *Wien klin. Woch.*, 1905.
Seiler, *Zeit. f. phys. Chem.*, 1907.
Strauss, *Centralb. f. inn. Med.*, 1872.
Tallqvist, *Zeit. f. klin. Med.*, 1903.
Teissier, quot. Blumenthal, *Path. d. Harnes*, 1903.
Vohl, *Arch. f. phys. Heilk.*, 1858.

APPENDIX

GENERAL PROPERTIES AND REACTIONS OF THE SUGARS AND RELATED SUBSTANCES

Specific Gravity of Saccharine Solutions.—The specific gravity of a saccharine solution depends chiefly on the amount of dissolved solid, and is approximately equal for equal strengths of different carbóhydrates. Solutions of dextrose have a slightly lower specific gravity than the corresponding solutions of cane-sugar, while solutions of levulose, maltose, and invert sugar give slightly higher readings.

Fermentation.—Yeasts, and certain other lowly organisms, when placed in a saccharine fluid, and kept under suitable conditions, are capable of rapid multiplication and exert a peculiar chemical change, known as alcoholic fermentation, in the mass of the sugar, which is broken up into alcohol and carbon dioxide. This decomposition is not the direct result of the changes in the plant protoplasm, but is a side issue of its life processes resulting from the action of an enzyme or ferment which it produces. By careful experiment it has been shown that only about 1 per cent. of the sugar is used as food by the yeast cells in their growth, and that the actual amount of carbon dioxide they expire is very small. In the case of dextrose the chemical changes induced by alcoholic fermentation may be represented :—

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$
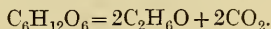
Theoretically, 100 parts of dextrose should yield 51·11 parts by weight of ethyl alcohol and 48·89 parts of carbon dioxide, but it was shown by Pasteur that only 48·5 per cent. of alcohol and 46·5 per cent. of carbon dioxide actually result. In addition from 2·5 to 3 per cent. of glycerine, 0·4 to 0·7 per cent. of succinic acid, and 0·8 to 1·3 per cent. of other substances, including amyl alcohol, isobutyric alcohol, propyl alcohol, and traces of fatty acids and lactic acid, are formed.

The ease with which the different sugars undergo fermentation varies considerably, and is partly dependent upon the variety of yeast employed, and partly on the structure of the sugar. Grape-sugar, fructose, mannose, and invert sugar are fermentable by all yeasts, but cane-sugar, maltose, and milk-sugar are only fermented after inversion by dilute acids or appropriate enzymes, which for cane-sugar and maltose are contained in ordinary yeast. The pentoses are not fermented by any known yeast, but they are attacked and slowly broken down by bacteria. It has been found that any species of yeast which ferments one of the three sugars, glucose, mannose, and fructose, also ferments

the others, and with approximately the same readiness. These three hexoses are closely related to each other in structure, and it is possible to convert them one into the other by treatment with alkalies. In the transformation it is assumed that a common, or enolic, form acts as an intermediate substance. Similarly in the process of fermentation it is believed that the first step is the conversion of the sugar into this common form by an enzyme contained in the yeast. Subsequent action of this, or another, enzyme in the yeast causes simplification of the molecule, the breakdown commencing at the linkage between the terminal carbon atoms. Galactose is fermented with much greater difficulty than glucose, and many varieties of yeast do not act upon it at all. No yeast is known, however, which is capable of fermenting galactose which does not also ferment glucose. It is believed that this variation in susceptibility to fermentation is dependent upon a difference in the configuration (*i.e.* the position of the hydroxyl, -OH, groups relative to the chain of carbon atoms) in the two sugars which, while not sufficient to prevent fermentation altogether, makes galactose far more resistant to attack. It is probable that galactose is fermented by a different mechanism, and that the enzyme which converts it to the enolic form is less widely distributed in yeasts than that which produces the change in glucose, mannose, and fructose. This hypothesis is supported by the fact that the isomerides of galactose, talose, and tagatose are not fermented by any yeast whose action toward them has up to the present been investigated, and presumably no yeast exists capable of converting them into the enolic form. Many other substances which are closely related to glucose, such as gluconic acid, glucuronic (glycuronic) acid, the methyl glucosides, &c., are not fermented by yeast, although the greater part of the molecule is the same. The shortening of the carbon chain to five atoms in the pentoses also appears to be sufficient to place the sugar molecule out of harmony with the yeast enzyme, and thus prevent its disruption. From these, and other, considerations it appears highly probable that there is a very close relationship between the configuration of a fermentable sugar and the enzyme which causes its fermentation. Armstrong has aptly compared the relationship to that which exists between the fingers of a glove on the hand ; it is impossible to fit the glove if the position of the fingers is altered, and, moreover, a right-handed glove will not fit the left hand.

In testing a saccharine fluid for fermentation it is necessary that certain points should be attended to—(1) the solution should not be too concentrated, over 10 per cent. ; (2) it should be neutral, or faintly acid, in reaction, never alkaline ; (3) no free antiseptic should be present ; (4) the yeast should be fresh and free from starch ; (5) the addition of a little yeast ash, or sodium phosphate and potassium tartrate, is advisable with pure solutions to provide material for the growth of the yeast ; (6) a blank experiment should be conducted to prove that the yeast by itself does not give off carbon dioxide under the conditions of the experiment ; (7) the fermenting fluid should be kept at a temperature of $25°$ to $35°$ C. ($77°$ to $95°$ F.).

Optical Characters—Polarisation.—If a piece of the semi-transparent mineral tourmaline is cut into slices by sections parallel to its axis, and one of these slices is laid upon another, it is found that in certain positions they form an opaque combination, while in others they are transparent. The combination is most transparent in two positions, one when the slices lay in the natural relation they occupied in the crystal, and the other when one of the slices is rotated through 180°, and is most opaque in two other positions at right angles to these. The light that has passed through one such plate is in a peculiar condition, and, since it contains rays that vibrate in one plane only, is said to be plane-polarised. Polarised light cannot be distinguished from ordinary light by the naked eye, but is shown by the interference to its passage caused by the interposition of another " polariser," called the " analyser," with its axis at right angles to the first.

In the above system the plate of tourmaline next the eye is the analyser, and the other plate the polariser. One of the most convenient and effective contrivances for polarising light, or analysing it when polarised, is that known, after its inventor, as Nicol's prism. This is made by splitting a rhomb of Iceland-spar, or calc-spar, along a diagonal plane, and cementing the two pieces together in their natural position with Canada balsam. Iceland-spar, in common with certain other substances, shows the phenomenon of double refraction— that is to say, an incident beam of light gives rise to two refracted rays which take different paths, one of these rays is termed the " ordinary," the other the " extraordinary " ray. In the Nicol's prism the ordinary ray is totally reflected on meeting the first surface of the Canada balsam and passes out at one side of the prism, while the extraordinary ray is transmitted through the balsam, and emerges at the end of the prism parallel to the direction of the incident beam, but polarised. This apparatus has nearly all the advantages of a tourmaline plate, with the additional advantage of much greater transparency and of complete polarisation.

When a plate of quartz, cut perpendicular to the axis, is interposed between a polariser and an analyser colour is exhibited, the tints changing as the analyser is rotated. Similar colour effects are produced when the solution of sugar, enclosed in the tube with plane glass ends, is substituted for the quartz. If homogeneous light, such as that from a sodium flame, is employed, it is found that, if the analyser is first adjusted to produce total extinction of the polarised light and the quartz or saccharine fluid is then introduced, there is partial restoration of light. On rotating the analyser through a certain angle, depending in the one case on the thickness of the quartz plate and in the other on the length of the tube and the strength of the solution, there is again complete extinction of light. The action thus exerted is called " rotation of the plane of polarisation." In the case of ordinary quartz and solutions of certain substances, it is necessary to rotate the analyser in the direction of the hands of a watch as seen by the observer, the rotation of the plane of polarisation is then

said to be right-handed, and the substance to be " dextro-rotatory." In cases of left-handed quartz and solutions of certain other substances, the rotation of the plane of polarised light is in the opposite direction, and the substance is said to be " levo-rotatory."

The power of rotating polarised light possessed by a particular sugar is, under certain circumstances, a fixed quantity, known as its " specific rotation," and as this property is also exerted by solutions of the sugars, the angle through which rotation occurs serves for their accurate estimation.

The specific rotation of a substance may be defined as the amount of rotation, in degrees of a circle, of the plane of polarised light pro-duced by 1 gram of the substance, dissolved in 1 c.c. of liquid, enclosed in a tube 1 decimetre long. The reading is usually taken at 30° C. and by homogeneous yellow (Na) light. It is necessary to make the measure-ments with monochromatic light of one particular wave-length, as the apparent specific rotatory power of a substance varies greatly with the wave-length of the light employed. It is most usual to refer the rotation to the D line of the spectrum, the rotation being expressed as $[a]_\mathrm{D}$.

The optical rotatory power of the freshly made solution of most sugars undergoes a change on standing, sometimes increasing, but more generally falling, until a constant value is reached. This pheno-menon, which is now known as " mutarotation," or " multirotation," was formerly termed birotation, because the rotatory power of dextrose in a fresh solution is nearly twice as great as it eventually becomes. Mutarotation is due to the fact that most sugars exist in solution as a mixture of two forms in equilibrium. Thus, solid dextrose is the a-modification of high rotatory power which does not persist as such in the freshly made solution, but slowly passes over in part into a β-form of lower rotatory power. The change takes place very slowly when highly purified materials are used, but is much accelerated by impurities, and takes place almost immediately if a trace of an alkali is added to the solution.

Melting-point. — The carbohydrates seldom melt sharply, be-cause fusion is nearly always preceded by slight decomposition. Their melting-points are therefore of minor importance as specific properties. The instability of the sugars towards heat is also manifested by their tendency to pass into the state of uncrystallisable syrups when their solutions are concentrated by boiling under the ordinary atmospheric pressure.

The Action of Alkalies.—All the monosaccharides are readily decomposed by alkalies. On being heated with a caustic alkali, such as sodium, potassium, or ammonium hydrate, a solution of a mono-saccharide sugar, such as dextrose, turns brown at about 60° C., and is entirely decomposed by prolonged boiling into a variety of sub-stances, including lactic acid, formic acid, and various aldehydes. Alkaline salts, such as sodium or potassium carbonate, have a similar,

but less intense, action. Cane-sugar is not affected by dilute solutions of caustic alkalies, or alkaline carbonates, in the cold, and only very slowly on heating. It is decomposed, however, by being boiled with a strong alkaline solution. Fused with solid caustic potash it gives rise to potassium oxalate, acetate, &c.

The Action of Oxidising Agents.—The reducing sugars which contains an aldehyde group are easily oxidised by bromine in the presence of water, and give rise to monobasic acids by a transformation of their terminal -CHO group into carboxyl :—

$$R.CHO + H_2O + Br = R - COOH + HBr .$$
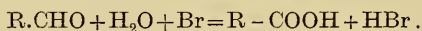
Thus xylose gives rise to xylonic acid, glucose to gluconic acid, mannose to mannonic acid, and galactose to galactonic acid, the last three being stereo-isomers of the same formula, $C_5H_6(OH)_5.COOH$. In some instances (*e.g.* xylose) this reaction can be utilised to differentiate the sugar.

Action of Concentrated Mineral Acids.—Concentrated *nitric acid* in the cold combines with sugars to form nitric esters.

When heated with moderately concentrated nitric acid the sugars undergo oxidation, giving rise to acids which differ accordingly to the nature of the sugar and the concentration of the acid. The *aldoses* are oxidised at each end of the chain, and yield di-basic acids with the general formula, $COOH(CH.OH)_nCOOH$.

Thus when dextrose (2 grams) is heated with nitric acid of a density of 1·2 (10 c.c.), evaporated to a syrup, dissolved in water (5 or 6 c.c.), the solution saturated with potassium carbonate and acidified with glacial acetic acid (3 or 4 c.c.), white, transparent, needle-like crystals, arranged in rosettes, or singly, of acid saccharate of potassium separate out on cooling, owing to the formation of saccharic acid, by the action of the nitric acid on the dextrose. If galactose is treated in a similar way mucic acid is formed, and can be readily separated by its insolubility in water. Lactose being a di-saccharide yielding dextrose and galactose, gives rise to a mixture of saccharic and mucic acid. The latter can be separated from the former by its insolubility in water, appearing as short prisms, and can be distinguished from calcium oxalate by its complete solubility in ammonia. The formation of mucic acid is characteristic of galactose, and substances containing it, and is therefore used to detect their presence. The *ketoses* on being heated with nitric acid break down and yield acids poorer in carbon. Thus levulose forms oxalic acid, tartaric acid, acetic acid, formic acid, &c.

Dextrose dissolves in cold *sulphuric acid* to form dextrose sulphonic acid, without undergoing any colour change. Cane-sugar, on the other hand, is readily carbonised by concentrated sulphuric acid, forming a bulky black mass, with the evolution of sulphur dioxide and other volatile products.

Action of Dilute Mineral Acids.—All the di- and poly-saccharides when heated with dilute mineral acids undergo hydrolysis

or inversion—that is to say, they are decomposed into the simple monosaccharides from which they are derived. A solution of cane-sugar, for instance, when heated with dilute hydrochloric, or sulphuric acid, gives rise to a mixture of dextrose and levulose. In the process the specific gravity of the solution is raised, the sugar loses its power of crystallising readily, and its optical activity is changed from right to left-handed—that is to say, it is " inverted." This change in optical activity is due to the levo-rotatory power of the levulose formed being greater than the dextro-rotatory power of the dextrose, which is present in equal amount. Although a similar change in rotatory power does not necessarily follow the hydrolysis of other di-saccharides, &c., the term inversion is frequently applied to the process generally. It is better, however, to speak of it as hydrolysis, for it is attended by the assimilation of the elements of water :—

$$C_{12}H_{22}O_{11} + H_2O_0 = C_6H_{12}O_6 + C_6H_{12}O_6 \, .$$
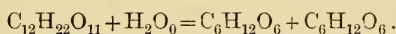
The rate at which hydrolysis, or inversion, takes place depends upon the nature of the acid, its concentration, the temperature, and the nature of the sugar. Cane-sugar is most readily inverted, maltose much less readily, and lactose a little less readily than maltose. Cane-sugar is inverted by boiling with citric acid, 2 per cent., but lactose is unaffected. When the reducing powers of a sugar before and after inversion are to be compared, the acid solution must be made neutral, or nearly neutral, with sodium carbonate.

Action of Organic Acids.—With organic acids sugars form ethereal salts, or esters. The most important of these is the benzoyl ester, which is particularly useful in isolating dextrose and other carbohydrates from physiological and pathological fluids. If a solution of dextrose is shaken with 6 parts of benzoyl chloride and 48 parts of a 20 per cent. solution of sodium hydrate for each part of dextrose in the solution, until the smell of benzoyl chloride has disappeared, it forms dextrose pentabenzoate, which crystallises out on cooling the fluid on ice and standing for twenty-four hours. If the benzoate is then separated off, dissolved in alcohol, and recrystallised, it appears as colourless needles with a melting-point of 179° C.

Reducing Properties.—With the exception of cane-sugar and raffinose, all the commonly occurring sugars show a strong tendency to undergo oxidation, and therefore act as reducing agents. This property depends upon the presence of an active carbonyl group. It is consequently not specific of the sugars, but is shared by all substances having the properties of aldehydes and ketones. Bismuth, mercury, silver, platinum, and gold salts are reduced to the metallic state by hot alkaline solutions of the reducing sugars, and some reduce ammoniacal silver nitrate even in the cold. Cupric and ferric salts are converted into cuprous and ferrous compounds, while picric acid is converted into picramic acid, ferricyanides to ferrocyanides, and various dyes, such as indigotin, are reduced to colourless compounds by heating with alkaline solution of these sugars.

The reaction that occurs when a solution of a reducing sugar is heated with an oxidising agent in the presence of a caustic alkali is complex, and is only imperfectly understood. The principal products are said to be formic, oxalic, glycollic, and carbonic acids, but the products of the alkali itself on the sugar have also to be taken into account. The non-reducing sugars show the same reactions after hydrolysis by heating with acids, or through the action of enzymes. If a solution of cane-sugar, for instance, is heated for five minutes, or longer, in a boiling water bath with one-twentieth of its volume of concentrated hydrochloric acid, and is then cooled, neutralised with soda solution, and tested, it will be found to reduce alkaline solutions of the metals, &c.

Colour Reactions.—When a reducing sugar is heated with a concentrated mineral acid it yields furfurol, which can be detected by the formation of coloured condensation products with various substances of the phenol group. The colour obtained depends on the variety of sugar, and upon the phenol employed.

Molisch's Test.—About 5 mg. of the substance to be tested are placed in a narrow test-tube, and dissolved in 10 drops of water. Two drops of a 10 per cent. chloroform solution of a-naphthol are added, and the contents of the test-tube well mixed. One c.c. of pure concentrated sulphuric acid is allowed to flow down the lower inclined side of the tube so that it may form a layer beneath the aqueous solution, without mixing with it. In the presence of a carbohydrate a red ring will appear at the line of junction within a few seconds. On standing the colour soon changes to a dark purple. If the tube is shaken, and allowed to stand for one or two minutes, and the contents then diluted with 5 c.c. of cold water, a dull violet precipitate will immediately appear if a carbohydrate is present. The addition of an excess of strong ammonia changes the colour to a rusty yellowish-brown. Any substance that gives dull violet and rusty brown precipitate, as well as the purple coloration, under the circumstances described, may be assumed to be a carbohydrate. It is essential, however, that the substance should be free from all traces of filter-paper, particles of wood, dust, &c., as the test is extremely delicate. The purity of the reagents employed should also be beyond all question, and it is most important that the sulphuric acid should be free from all traces of nitrous acid. It is advisable to conduct a blank experiment by shaking 1 drop of the a-naphthol solution with 10 drops of water and 1 c.c. of the sulphuric acid, when the mixture should be of a golden yellow colour. If it is dark green the reagents are not sufficiently pure. The naphthol solution does not keep well, and should be prepared as required. Most albumens give the violet coloration with Molisch's reaction, owing to the presence of a carbohydrate group, but do not give the violet and rusty brown precipitates. Casein does not react with Molisch's test.

Phloroglucine Test.—Shake an excess of phloroglucine with a mixture of equal parts of concentrated hydrochloric acid and water until the solution is saturated. Boil 3 c.c. of this reagent with about 0·03 gram of the carbohydrate in a small test-tube. Note the colour when it just commences to boil. Continue to boil until the colour darkens considerably and the solution begins to appear slightly

turbid, usually within about one minute. Pour the hot solution, without delay, on to a wet filter-paper, and rinse the scanty precipitate with a little cold dilute alcohol. Note the colour of the precipitate while moist. With arabinose and xylose the first coloration on heating is a pure red to violet-red, but it rapidly intensifies and darkens as the heating is continued. The colour of the precipitate varies, according to the duration of the boiling, from very dark purple to black, if the heating has been continued too long. If the precipitate is dissolved in alcohol, and the solution is examined with the spectroscope, an absorption band is seen in the green should a pentose be present. With fructose, rhamnose, and sorbinose the first coloration is a yellow-orange, which quickly passes through dark orange to dingy brown. The precipitate is of a rusty brown, or dark shade of yellow-orange, or orange, which may be easily changed to a dull black if the boiling is too long continued. Galactose and lactose give a similar colour change, but on spectroscopic examination they do not show the sharp absorption bands. Solutions of glucuronic acid give a similar colour reaction, and show the same absorption bands as the pentoses.

Orcin Test.—About 0·3 to 0·4 gram of the carbohydrate, 2 or 3 c.c. of pure concentrated hydrochloric acid, and a few milligrams of orcin are mixed in a test-tube, and gently heated. At first a faint yellow tint is seen, but in a few seconds a violet-blue colour appears if a pentose is present, while the methylpentoses and hexoses give an orange-red coloration. On continuing the heating the colour intensifies, and finally a precipitate appears. If the contents of the test-tube are now cooled somewhat, and extracted with amyl alcohol, the extract on being examined with the spectroscope shows a distinct band between C and D (red and yellow), with often a second band in the red if pentoses are present. A band in the green may be seen if too much orcin has been used. Other sugars show no absorption bands, but glucuronic acid gives the same results as a pentose. If the solution is heated too rapidly the characteristic colour may be obscured by the darkening of the liquid, and by partial destruction of the sugar, with the formation of brown humous substances. This reaction also takes place at ordinary temperatures, but only after standing for several hours. If the hydrochloric acid contains iron the pentoses give a green, instead of a violet-blue, coloration.

Aniline Acetate Test.—Dissolve about 0·3 to 0·4 gram of the carbohydrate in 5 c.c. of dilute hydrochloric acid (1 vol. of HCl, sp. gr. 1·2, with 3 vol. of water). Boil for one minute in a test-tube. Insert a roll of thick filter-paper which has been soaked in mixture of 5 c.c. of aniline and 10 c.c. of 50 per cent. acetic acid and pressed between blotting-paper until only just moist, into the upper inch or so of the test-tube, and continue the boiling. Arabinose, xylose, fructose, rhamnose, and sorbinose give sufficient furfurol when treated in this way to turn the test-paper a bright pink. The other carbohydrates do not bring about any noticeable coloration. Xylidine acetate may be substituted for aniline acetate in this test, and is somewhat more sensitive.

Resorcin (Seliwanoff's) Test.—All carbohydrates when heated with resorcin and strong hydrochloric acid give a red coloration, due to the formation of furfurol and its condensation by the resorcin, but if the hydrochloric acid is diluted with its own volume of water the reaction is only given by the ketoses. On this fact is based the reaction of Seliwanoff which distinguishes the ketoses, and

particularly levulose, from the aldoses. A solution of a few milligrams of resorcin in diluted hydrochloric acid (1 vol. strong HCl to 2 vols. of water) is warmed with a few milligrams of the carbohydrate, when, if levulose, or another ketose, is present a beautiful red colour appears, and a red precipitate settles on standing. If the solution is neutralised with sodium carbonate and extracted with amyl alcohol, the alcohol takes on a red-green fluorescence, which, on the addition of a little absolute alcohol, becomes pure red. Examined with the spectroscope a band is seen between E and *b*, and in highly concentrated solutions a second band in the blue near F.

Rubner's Test.—When a solution of a reducing sugar is boiled for several minutes with a solution of acetate of lead and ammonia, a colour, varying from yellow to copper red, is produced. Under certain conditions the particular colour is characteristic, and may be used to distinguish certain sugars, and particularly lactose. It is in the first place essential that the sugar solution should not be too strong (0·5 to 1 gram per litre) ; secondly, too great heat should not be applied or a non-characteristic brown colour develops ; and thirdly, an excess of ammonia should be avoided, as it ruins the test.

To 10 c.c. of a solution of lactose of a strength of about 0·1 per cent. add about 1 gram of crystallised acetate of lead, and heat gently to dissolve the acetate. Now add ammonia drop by drop, shaking after each addition. The precipitate formed at first dissolves, but finally persists. The addition of ammonia should be continued until the liquid is distinctly turbid (usually about 1 to 2 c.c. of ammonia are necessary). The mixture is now boiled for two or three minutes, when a rose to orange colour develops. On standing for a few seconds a bright rose-coloured precipitate settles down, and the supernatent fluid appears orange to rose-coloured.

On carrying out the test with a solution of dextrose of the same strength, a white to yellowish precipitate is formed, and the fluid appears a clear yellow. With more concentrated solutions of the sugars it is necessary to increase the proportion of lead acetate, and consequently of ammonia. If the solution after the addition of ammonia is only warmed to 80° C. in a water-bath, dextrose gives a red solution with a rose or salmon-pink precipitate, lactose a yellow coffee brown or red coloration but no precipitate according to the concentration, maltose a slight yellow colour, and levulose no colour at all.

Combinations with Hydrazines.—Phenylhydrazin, and the substituted phenylhydrazins—

Phenyl-hydrazin	.	.	.	.	.	$C_6H_5.NH.NH_2$
Methyl-phenylhydrazin		.	.	.	.	$C_6H_5(CH_3)N.NH_2$
Benzyl-phenylhydrazin		.	.	.	.	$C_6H_5(C_7H_7)N.NH_2$
Di-phenylhydrazin	.	.	.	.	.	$C_6H_5(C_6H_5)N.NH_2$
Para-brom-phenylhydrazin	.		.	.	.	$C_6H_5(C_6H_4Br)N.NH_2$, &c.

form two series of compounds with sugars containing an active aldehyde, or ketonic, group—(1) the phenylhydrazones, in which one molecule of sugar combines with one molecule of the hydrazin ; and (2) the phenylosazones, in which one molecule of the sugar combines with two molecules of the hydrazin. Owing to the varying physical

and chemical properties of these compounds in the case of different sugars, they furnish a most important means for separating and identifying them.

1. *The hydrazones* are prepared by digesting the sugar, dissolved in not too much water, with the calculated quantity of phenylhydrazin, dissolved in its own weight of acetic acid, in the cold. The amount of phenylhydrazin required is deduced from the results of an approximate titration of the sugar solution. The most readily prepared is mannose phenylhydrazone, which is formed by allowing for each 180 grams of the sugar 108 grams of the base :—

$$CH_2OH.(CHOH)_4.CHO + NH_2.NH.C_6H_5 = CH_2OH.(CHOH)_4.CH:N - NH - C_6H_5 + H_2O$$
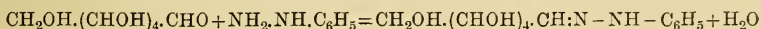
Preparation of Mannose-phenylhydrazone.—To 50 c.c. of a solution of mannose, of approximately 2 per cent. strength, add 6 to 7 c.c. of a solution containing 10 grams of phenylhydrazin and 10 c.c. of glacial acetic acid made up to 100 c.c. with water, and shake. The liquid becomes cloudy and forms a white deposit of the phenylhydrazone, which, under the microscope, is seen to consist of sphæro-crystals. They have a melting-point of 186° to 188° C.

The simple phenylhydrazones of the other sugars, with the exception of the rare sugars rhamnose and fukose, are readily soluble in water, and cannot therefore be easily prepared.

The substituted phenylhydrazins form hydrazones which are generally more insoluble than the corresponding simple hydrazones. They are therefore of considerable value in the detection and separation of the sugars.

Methyl-phenylhydrazin ($C_6H_5(CH_3).N.NH_2$) is chiefly useful in the recovery and separation of *galactose*, with which it forms an insoluble crystalline compound, melting at 180° C.

Benzyl-phenylhydrazin ($C_6H_5(C_7H_7).N.NH_2$) on being heated with a 96 per cent. alcoholic solution of the sugar gives a hydrazone which can be recovered by evaporating and recrystallisation from alcohol. *Dextrose* yields a benzyl-hydrazone which is levo-rotatory, and has a melting-point of 165° C. (171° to 172°). It is decomposed by boiling water into its constituents, dextrose and benzyl-phenylhydrazin. *Levulose* gives a similar compound, which is not, however, decomposed by boiling water, thus affording a means of differentiating the two sugars. *L-arabinose* and *galactose* both give benzyl-hydrazones, the former being insoluble in alcohol and having a melting-point of 170° to 174° C., the latter being feebly soluble and having a melting-point of 154° C.

Di-phenylhydrazin ($C_6H_5(C_6H_5)N.NH_2$) is only feebly soluble in water, so that in preparing the hydrazone it is necessary to dissolve the reagent in alcohol. This solution is heated with the sugar for two hours, or left in the cold for two to three days. The di-phenylhydrazone of *r-arabinose* melts at 204° to 205° C., the *xylose* compound at 107° to 108° C., the *dextrose* at 161° to 162° C., *mannose* at 155° C., *galactose* at 157° C. Levulose does not yield a crystallisable hydrazone with di-phenylhydrazin.

Para-brom-phenylhydrazin ($C_6H_5(C_6H_4Br).N.NH_2$) is a most important reagent for distinguishing arabinose from xylose and dextrose. *L-arabinose* forms a very feebly soluble para-brom-phenylhydrazone with a melting-point of 162° C., whereas the other two sugars do not yield an insoluble crystallisable hydrazone. *Glucuronic acid* also reacts with para-brom-phenylhydrazin to form a crystalline compound, insoluble in alcohol, which in the pure condition melts at 236° C., but when freshly prepared from the urine melts at 200° to 216° C.

Melting-points of the Hydrazones

	l arabinose.	r-arabinose.	l-xylose.	Dextrose.	Levulose.	Mannose.	Galactose.	Maltose.	Lactose.
Phenylhydrazone . .	150–153	..	..	144–146	144–146	186–188	158	130	..
Methyl-phenylhydrazone .	161–164	173	108–110	130	116–130	178	180–188	..	..
Benzyl-phenylhydrazone .	170	185	93	165	165	165	154–158	..	128
Di-phenylhydrazone . .	216–218	204–205	107–108	161–162	..	155	157	..	..
Para-brom-phenylhydrazone	160–162	160	128	147	147	208–210	168	..	..

The hydrazones are important, not only because of their physical characters, which lend themselves to the differentiation of the sugars, but also from the fact that when they are treated with certain reagents they are decomposed into the sugar and the hydrazin from which they were derived. Thus a phenylhydrazone treated with concentrated hydrochloric acid yields phenylhydrazin hydrochloride, and the sugar. After the excess of hydrochloric acid has been neutralised with lead carbonate, the sugar can be recovered from the solution. It is better, however, to bring about the decomposition with benzaldehyde.

The hydrazone is placed in a flask, provided with a reflux condenser, mixed with about a quarter more benzaldehyde than is theoretically required to bring about the decomposition, and four times the weight of a mixture of equal parts of water and strong alcohol, and heated on a water-bath for a half to one hour. It is then cooled, and filtered from the precipitated benzaldehyde phenylhydrazin. The filtrate is shaken with 2 volumes of ether, to remove the excess of benzaldehyde, decolorised with animal charcoal, and the sugar crystallised out.

$$C_6H_{12}O_5 : N.NH.C_6H_5 + C_6H_5COH = C_6H_5CH : N.NH.C_6H_5 + C_6H_{12}O_6$$
Mannose phenyl-   Benzal-   Benzaldehyde   Mannose.
hydrazone.   dehyde.   phenylhydrazin.
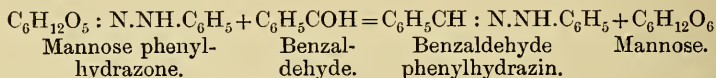
The substituted phenylhydrazins are similarly decomposed by formaldehyde. To bring about the decomposition the hydrazone is mixed with 35 per cent. formaldehyde and a little alcohol, to aid solution, and heated in a flask, provided with a reflux condenser, on

a water-bath, for about an hour. It is then cooled, extracted with ether several times, decolorised with animal charcoal, and evaporated, with the addition of a little water, until the excess of formaldehyde is driven off. The sugar is then crystallised out.

$$C_5H_{10}O_4N.N(C_6H_5) + CH_2O = CH_2.N.N(C_6H_5)_2 + C_5H_{10}O_5$$

| Arabinose de-phenylhydra-zone. | Formal-dehyde. | Formaldehyde de-phenylhy-drazin. | Arabinose. |
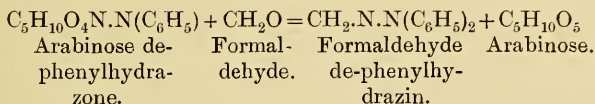
Osazones.—All the reducing sugars give with phenylhydrazin, in the presence of an excess of phenylhydrazin acetate, more or less insoluble yellow crystallisable compounds to which the name osazones has been given. Two molecules of the base react with one molecule of the sugar to form the osazone, hydrogen being liberated at the same time. The latter is not, however, set free, but reacts with the excess of phenylhydrazin, giving rise to aniline and ammonia. Thus with dextrose :—

$$C_6H_{12}O_6 + 3NH_2.NH.C_6H_5 = C_6H_{10}O_4.(N.NH.C_6H_5)_2 + 2H_2O + C_6H_5NH_2 + NH_3$$

| Dextrose. | Phenylhydrazin. | Dextrosazone. | Water. | Aniline. | Ammonia. |
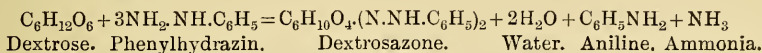
This reaction takes place in the cold, but occurs much more quickly on heating.

Preparation of Glucosazone.—Heat in a water-bath for one hour a mixture of 50 c.c. of a solution of dextrose, about 1 per cent., with 10 c.c. of a solution consisting of phenylhydrazin 10 grams, glacial acetic acid 10 c.c., and water to 100 c.c. Cool, and pour on to a moist filter. Wash the crystalline deposit with cold distilled water, and afterwards with methyl alcohol several times, using about 20 c.c. Dry by pressing between clean filter-paper, and take the melting-point. This procedure is only applicable to those osazones which are feebly soluble in methyl alcohol, such as glucosazone and maltosazone. Those which are more soluble in water and organic solvents, like maltosazone and lactosazone, must be purified by dissolving in boiling water and recrystallising several times.

The original method of preparing the osazones described by H. Fischer (*Ber. d . deutsch. chem. Ges.*, xvii. 579, 1884) may also be followed with the pure sugars. In this 1 gram of the sugar, 2 grams of phenylhydrazin hydrochloride, 3 grams of crystallised sodium acetate, and 20 grams of water are mixed together, and heated in a boiling water-bath for three-quarters to one and a half hours.

The rate at which osazone formation takes place is very different for the various sugars. Working with the pure substances and a definite mode of procedure advantage may be taken of this fact to roughly differentiate them (Marquenne). (See Mulliken, *Identification of Pure Organic Compounds*, p. 32.)

Place in a dry test-tube, having an internal diameter of 13 mm., 0·1 gram of the sugar, 0·2 gram of pure phenylhydrazin hydrochloride, 0·3 gram crystallised sodium acetate, and 2 c.c. of water. Close the tube loosely with a cork, to prevent evaporation, and stand in a tall narrow beaker containing two or three inches of water that is already briskly boiling. Note the exact moment of

immersion. Shake the tube occasionally, without, however, removing it from the beaker. If a precipitate finally separates, note the number of minutes that have elapsed up to the moment of its appearance. Note also whether the precipitate is white, yellow, or orange-yellow, and whether it is crystalline, flocculent, or tends to rise to the surface in oily drops.

Under the above-mentioned conditions the monosaccharides (pentoses and hexoses) all give precipitates which separated out from the hot liquid in a half to twenty minutes. *Mannose* gives a nearly white crystalline deposit in a half minute; *levulose* a yellow precipitate in two minutes; *dextrose* a yellow osazone which separates out suddenly in four to five minutes; *xylose* a light yellow osazone in seven minutes; *arabinose* an orange-yellow precipitate, which may, however, appear partly as brownish-yellow oily drops unless the sugar is very pure, in ten minutes; and *galactose* a yellow to orange-yellow osazone in fifteen to nineteen minutes. Of the di-saccharides some, like *maltose* and *lactose*, give products which do not separate out until the hot solution has cooled, while others like *saccharose* are gradually hydrolised to monosaccharides, which then give the corresponding osazones, but naturally require a longer time for the reaction than when the simple sugar was originally present. Saccharose begins to show a yellow osazone formation after about thirty minutes' heating. With *raffinose* no osazone separates out until the solution has been heated for about sixty minutes. The precipitates are all phenylosazones, except that from mannose, which is a simple hydrazone and is easily distinguished from the others by being white instead of yellow. Variations of a minute or two from the times stated will occasionally occur, and must be allowed for when selecting between two species of sugar whose values lie close together.

The osazones are all more or less insoluble in cold water, and some are only feebly soluble in hot water. They are easily soluble in hot 60 per cent. alcohol, but can be recrystallised out on boiling off the alcohol. They are also soluble in hot acetic acid. They are insoluble in ether, chloroform, benzol, and ligroin, but are soluble in pyridine, and a mixture of pyridine and alcohol.

The osazones generally crystallise well. They present considerable variations in form, which, to a certain extent, is dependent upon the conditions under which crystallisation occurs, but under the same conditions they present forms which are more or less characteristic. Examined under the microscope with a magnification of about 500 diameters, glucosazone crystals are seen as sheaves and bundles of yellowish-green crystals. Levulosazone crystals are similar, but are often somewhat coarser. Lactosazone appears as long, narrow, yellow plates, free or grouped in various shapes. Arabinosazone forms long, thin, hair-like needles which are curved and bent. Xylosazone is seen in long, straight needles. Maltosazone appears as short, leaf-like crystals which are very often grouped in rosettes.

The melting-point of the osazones of the different sugars is a con-

stant physical character which assists in their differentiation. It has not, however, the great importance that is sometimes attributed to it, especially in the case of osazones which melt at nearly the same temperature. Many osazones undergo decomposition in the neighbourhood of their point of fusion, with the result that the melting-point is lowered by the products of decomposition, and the more abundant these are—that is to say, the longer the heating is continued—the lower the melting-point appears to be. A constant reading, therefore, is only obtained when the determination is carried out as rapidly as possible, and at the minimum temperature for which the fusion-point is instantaneous. The presence of a very small quantity of impurity also lowers the melting-point very markedly.

Purification.—As a general rule the osazones may be purified by filtering off from the mother-liquor, well washing with cold distilled water, removing the excess of moisture by pressing between filter-paper, or by means of a suction-pump, dissolving in hot 60 per cent. alcohol, driving off the excess of alcohol with heat, and crystallising out. The osazone is then filtered off, and dried over sulphuric acid, or in an oven at a low temperature.

Melting-point Determination.—The usual method of determining the melting-point is to place a few milligrammes of the substance in a thin-walled glass capillary tube, sealed at the lower end, about 6 to 7 cm. long and with an internal diameter of about 1 mm. This is attached to a delicate thermometer by a spiral of fine platinum wire in such a way that the substance in the capillary tube is situated opposite the middle of the thermometer bulb. The thermometer is introduced into a small test-tube, with a diameter of about 15 mm., containing sufficient colourless sulphuric acid (sp. gr. 1·84) to cover the bulb. The test-tube is suspended by its flanged lip in a small round-bottomed flask with a bulb of about 65 mm. diameter, a neck 75 mm. long and a 20 mm. diameter, and a total capacity of about 200 c.c., which contains sulphuric acid up to the level of that in the test-tube. The flask is heated rapidly to within a few degrees of the approximate melting-point of the osazone, which can be determined by a trial experiment if necessary. It is then heated more cautiously, and the temperature just before complete liquefaction noted as the melting-point. It is a common practice to give as the melting-point the temperature at which the first drop of sufficient size to detach itself from the solid mass appears. Differences of several degrees between melting-points obtained by different methods of observation are therefore possible.

A method which is much used in France is the " bloc Maquenne." This is a block of metal heated by a gas flame, and provided with a thermometer which registers its temperature. The block is rapidly heated by the gas flame so as to have a rise of 3° to 5° C. per minute. At each rise of 5° C. a small quantity of the dry powdered osazone is thrown on to the surface of the block, and the temperature at which instantaneous fusion occurs is noted. The block is then cooled to a few degrees below this point, and gently reheated so as to have a rise of about 1° C. every three to four minutes. At each degree a small quantity of the osazone is again thrown on to the block, and the exact melting-point noted. This method gives readings which it is to be noted differ considerably from those obtained by the classical capillary tube method. Thus glucosazone

2 E

melts at 230° to 232° C. as compared with 204° to 205° C. by the ordinary method, and lactosazone at 213° to 215° C. as compared with 200° C. When considering the melting-point of an osazone it is obvious that, unless the product was quite pure and the exact method of determination is taken into account, the results may prove most misleading.

Solutions of the osazones are for the most part optically active, and this property serves for the further identification and separation of the sugars. If 0·2 gram of the pure osazone is dissolved in a mixture of 4 grams of pyridin and 6 grams of absolute alcohol, and examined in a 100 mm. tube with the polariscope, the following readings, according to Neuberg (*Ber. d. deutsch. chem. Ges.*, xxxii., 579, 1884), are obtained :—

Pentoses	l-arabinose phenylosazone	$+1·10°$
	do. para-brom-phenylosazone	$+0·28°$
	l-xylose phenylosazone	$-0·15°$
	do. para-brom-phenylosazone	$\pm0·00°$
	Rhamnose phenylosazone	$+1·24°$
Hexoses	d-glucose phenylosazone	$-1·30°$
	do. para-brom-phenylosazone	$-0·31°$
	fructose phenylosazone	$-1·30°$
	mannose phenylosazone	$-1·30°$
	d-galactose phenylosazone	$+0·48°$
Disaccharides	maltose phenylosazone	$+1·30°$
	lactose phenylosazone	$\pm0·05°$
Glucuronic acid para-brom-phenylhydrazin		$-7·25°$

The percentage of nitrogen contained in the pure osazone affords another means of differentiating the groups of sugars from each other ; thus the osazones of the *pentoses* yield 17·7 per cent. of nitrogen, the *hexoses* 15·7 per cent., the *disaccharides* 10·7 per cent., and the glucuronic acid compound of phenylhydrazin 16·4 per cent. The osazones must be very carefully purified, however, and the nitrogen be determined by Dumas' method, as the Kjeldahl process does not give satisfactory results.

When treated with strong hydrochloric acid the osazones are decomposed with the separation of phenylhydrazin hydrochloride, and the formation, not of the sugar, but substances known as osones containing the group $-CO.CHO$:—

$$C_4H_5(OH)_4.C(N_2HC_6H_5).CH.N_2HC_6H_5 + HCl + 2H_2O =$$
$$C_4H_5(OH)_4.CO.CHO + 2C_6H_5NH.NH_2,HCl$$
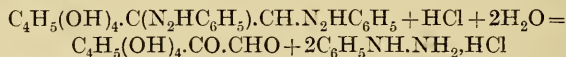
The osones may be reduced to sugars by the aid of zinc dust and acetic acid, but a given osazone does not necessarily yield the sugar from which it was derived ; thus glucosazone, mannosazone, and fructosazone all give rise to levulose :—

$$C_4H_5(OH)_4.CO. CHO + 2H = C_4H_5(OH)_4CO.CH_2.OH$$
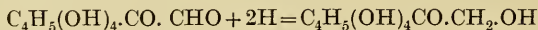
showing that they are not three separate osazones, and thus accounting for their identical physical properties.

Melting-points of the Osazones.

	l-arabinose.	r-arabinose.	l-xylose.	Dextrose.	Levulose.	Mannose.	Galactose.	Maltose.	Lactose.	Glucuronic acid.
Phenylhydrazone	160	166–168	152–170	204–205	204–205	204–205	194–195	202–20	210–212	(114–115 / 200–212)
Methylphenylhydrazone	...	...	...	142–153	158–160	...	...	...	...	...
Benzylphenylhydrazone	...	...	...	...	190	...	...	...	...	...
Para-brom-phenylhydrazone	196–200	200–202	208	222	222	...	...	198	...	236

Monosaccharides. — The general characters of the monosaccharides, and of the pentoses and hexoses, have already been considered. The properties of the individual sugars will now be briefly dealt with.

Pentoses.—_Arabinose_ (_Pectiose_) is most readily prepared from gum-arabic, or cherry-gum, by heating it on a water-bath with 2 per cent. sulphuric acid for ten to fifteen minutes. The product thus prepared is the dextro-rotatory l-arabinose. The form usually met with in the urine is the racemic, or optically inactive, r-arabinose. The former crystallises out as glancing bright needles, or prisms, which melt at 150° C., and, in solution, deflects polarised light to the right ($a_D =$ $+104°$ to $105°$). The latter crystallises in rhombic plate, or hard prisms, and melts at 164° to 165° C. Its solution has no action on polarised light.

Arabinose has a sweet taste, is easily soluble in water, but is only soluble with difficulty in alcohol, and is insoluble in ether. It reduces Fehling's and Nylander's solutions somewhat better than xylose, 10 c.c. of Fehling's solution being reduced by 43 mg. of arabinose. With phenyl-hydrazin it yields, after prolonged warming, an orange-yellow crystalline osazone, which, however, often separates out in the form of oily brownish-yellow drops, unless the sugar is very pure. Microscopically the osazone is seen to consist of sheaves of fine yellow flexible crystals, which when irrigated with 33 per cent. sulphuric acid dissolve, and disappear, a few seconds after the acid reaches them. The purified product melts at 157° to 158° C., but as prepared from the urine it more often melts at about 150° to 156° C. It contains 17·07 per cent. of nitrogen theoretically, but in practice the nitrogen content generally works

out at about 17·01 per cent. Alcoholic solutions of the osazone are at first dextro-rotatory ($a_D = +18·9°$.), but in a few hours become optically inactive. With di-phenylhydrazin it gives colourless needles, which are insoluble in alcohol and cold water, but are soluble in glacial acetic acid and pyridin, and melt at 204° to 205° C. Unlike xylose it yields a feebly soluble hydrazin compound with para-brom-phenylhydrazin, which melts at 200° to 202° C. Arabinose yields arabonic acid on oxidation.

l-xylose (*wood-sugar*) is prepared by the action of dilute acids on wood, gum, or straw. It can also be prepared from the nucleo-proteid of the pancreas, liver, and other organs of the body. It crystallises out in white needles or prisms, has a sweet taste, is soluble in water and hot alcohol, but is insoluble in cold alcohol and ether. The pure product melts at 153° to 154° C. It is dextro-rotatory ($a_D = +18·1°$), but shows strong mutarotation, the deviation depending upon the concentration of the solution. Like arabinose it is not fermented by yeast, but reduces Fehling's (10 c.c. = 45·6 mg. xylose) and Nylander's solutions, and gives an orange precipitate with Rubner's test. With phenylhydrazin it gives an orange yellow, crystalline osazone, consisting of long fine needles, which are readily soluble in 33 per cent. sulphuric acid. The osazone is easily soluble in alcohol and less easily in acetone. Its solution in alcohol is strongly levo-rotatory ($a_D = -43·4°$), and a solution in 4 per cent. acetic acid is also levo-rotatory ($a_D = -1·3°$), by which it is distinguished from the osazone of arabinose. On being rapidly heated the osazone melts at 159° to 160° C. With di-phenylhydrazin it forms a hydrazone that melts at 107° to 108° C. Xylose does not give a crystalline hydrazone with para-brom-phenylhydrazin. It is also distinguished from ara-binose by forming xylonic acid ($C_5H_{10}O_6$) on being oxidised with bromine. This can be separated out as a characteristic insoluble double salt of cadmium and bromine, by treating the product with cadmium carbonate. With brucine it forms a salt which melts at 172° to 174° C.

Hexoses.—*D-glucose, dextrose, or grape-sugar* is met with in asso-ciation with levulose (fructose) in the juices of grapes, and other ripening fruits. The two hexoses are probably derived by hydrolysis from pre-existing cane-sugar, with which they usually occur. Dex-trose can also be derived from other di-saccharides and polysaccharides, such as lactose or milk-sugar, maltose or malt-sugar, starch, and cellulose, by the action of dilute acids or appropriate ferments. In the animal body it occurs in small quantities in the blood and lymph, and in minimal traces in normal urine. It is the only hexose which does so exist normally ; levulose, mannose, and galactose on reaching the liver being transformed, with dextrose, into the polysaccharide glycogen. The urine of diabetics is characterised by a more or less marked increase of dextrose.

Dextrose separates from aqueous solutions with one molecule of water of crystallisation, but this is only loosely held, as the anhydrous substance may be crystallised from dilute alcohol. Unlike cane-sugar·

it never separates in well-defined crystals, but is usually met with as a crystalline powder. It is about half as sweet as cane-sugar. It is dextro-rotatory ($+52°$ 7′ at $20°$ C.), but a freshly made solution is more markedly dextro-rotatory (mutarotation). It is fermented by yeast within twelve to fifteen hours at $20°$ to $30°$ C., forming principally alcohol and carbon dioxide, but traces of fusel-oil, glycerol, succinic acid, &c., also appear. Heated with alkalies (Moore's test) a solution of dextrose turns brown, forming acetone, acetic, lactic, formic acids, &c. It reduces alkaline solutions of copper (Trommer's test, Fehling's test, &c.), bismuth (Nylander's test), and also acetic acid solutions of copper acetate (Barfoed's test). One molecule of dextrose always reduces exactly the same quantity (approximately 5 molecules) of cupric to cuprous oxide, a property which is generally used as the basis for estimating the quantity present in a given solution. With phenylhydrazin it forms a soluble hydrazone and an insoluble osazone, which suddenly separates out from the hot solution, after four or five minutes' heating, if at least 5 per cent. is present. The osazone forms a yellow precipitate, which, on microscopical examination, is seen to consist of coarse greenish-yellow crystals, and which on being irrigated with 33 per cent. sulphuric acid do not readily dissolve. The melting-point of the pure dry product is $204°$ to $205°$ C., but the crude preparation, as obtained from the urine, usually melts between $173°$ and $194°$ C. The pyridine solution of the osazone is levo-rotatory ($-1°$ 30′). The nitrogen content, by Dumas' method, is 15·7 per cent. With para-brom-phenylhydrazin it gives an osazone (melting-point $222°$ C.), with methyl-phenylhydrazin a hydrazone (melting-point $130°$ C.), and also hydrazones with benzyl-phenyl-hydrazin (melting-point $165°$ C.), and di-phenylhydrazin (melting-point $161°$ C.). Dextrose does not give the aniline acetate and other common tests for furfurol under ordinary conditions (*cf.* the pentoses). On oxidation with nitric acid it yields saccharic, but not mucic acid (*cf.* galactose). Oxidised with bromine it gives gluconic acid.

D-glucosamine or aminoglucose ($C_6H_{12}O_5NH_2$) was the first well-defined carbohydrate compound isolated from animal tissues (Ledderhose, 1878). It is of some physiological interest, as it is a constituent of mucins and mucoids, and, with glycuronic acid, enters into the composition of the chondroitin sulphuric acid of cartilage. It is most readily prepared by the action of concentrated hydrochloric acid on the chitin in lobster shells, or fungus cellulose, which gives the hydrochloride.

D-fructose or lœvulose is found with dextrose in fruit juices, honey, &c., the mixture being termed fruit- or invert-sugar. Combined with dextrose it occurs in cane-sugar, raffinose, &c., from which it can be prepared by the action of dilute acids and ferments. The polysaccharide inulin yields fructose alone when hydrolysed. It is met with in pathological urines, exudates, and transudates, rarely alone, generally with dextrose, and in normal and diabetic blood. The form met with in diabetic urine differs from plant levulose in being precipitated from its solution by basic lead acetate.

Lævulose crystallises less easily than dextrose, in long, fine, hygroscopic needles, or crusts, from alcohol, and in needles from water. It is very soluble in water, is soluble in five parts of cold absolute alcohol, and, unlike other sugars, it is soluble in ether. It tastes about as sweet as cane-sugar. With calcium it forms a feebly soluble, colourless compound $(C_6H_{12}O_6.Ca(OH)_2)$, by which it can be separated from the more soluble dextrose salt. It is levo-rotatory, and exhibits mutarotation, but is remarkable for the very large change produced in the specific rotatory power by alterations in temperature. The rotatory power falls (*i.e.* becomes less negative) as the temperature is increased. At 20° C. a 10 per cent. solution shows a rotation of $-90°$ to $-92°$, which is rather more to the left than dextrose is to the right, but at 82° C. it is equal and oppositive to that of dextrose. It gives the same reduction reactions as dextrose, but rather more rapidly. Half a gram of levulose in 1 per cent. solution reduces 97·2 c.c. of Fehling solution. With phenylhydrazin it forms an osazone with the same melting-point, nitrogen content, &c., as dextrose. Like other ketoses it gives with methyl-phenylhydrazin a characteristic compound, consisting of yellow needles that melt at 158° to 160° C. This osazone is insoluble in cold water, but is soluble in hot alcohol. A pyridin-alcohol solution is dextro-rotatory (0·2 gram in 10 c.c. = $+1·40°$). Levulose gives no crystalline compound with di-phenylhydrazin (*cf.* dextrose). Like dextrose, it is easily fermented by brewer's yeast within twelve to fifteen hours at 37° C., and forms the same products. Like other ketoses, it yields furfurol easily and in large quantities on being heated with hydrochloric acid, and on this fact is based Seliwanoff's test, in which the furfurol formed is recognised by the colour reaction it gives with phloroglucin. Arabinose and xylose give a similar reaction, but, while the first coloration with levulose is a yellow-orange, that quickly passes through dark orange to a dingy brown, the pentoses give on first heating a pure red to violet-red, which rapidly intensifies and darkens.

Dextrose and levulose may be recognised in a mixture of the two by the β-naphtholhydrazin reaction of Hilger and Rothenfusser, d-glucose forming a hydrazone with a melting-point of 117° C., and the levulose a hydrazone with a melting-point of 162° C. Levulose can also be detected in a mixture with other sugars by the blue colour which it strikes with a solution of ammonium molybdate and acetic acid on heating in a water-bath, other sugars giving a feeble green. On oxidation with nitric acid, levulose gives tartaric and glycollic acid.

D-mannose, or seminose, is widely distributed in nature, occurring in many plants in the form of anhydride-like condensation products, known as mannosans, which are converted into mannose on hydrolysis with dilute acids. In general properties it is very similar to d-glucose, being fermented by the same yeasts, exhibiting mutarotation, and forming the same osazone with phenylhydrazin. Its most characteristic reaction is the formation of a very sparingly soluble hydrazone with phenylhydrazin, which enables it to be easily identified. The

hydrazone is precipitated out in white, sphero-crystals in the cold within a few minutes, when phenylhydrazin is added to a solution of mannose and acetic acid. The hydrazone has a melting-point of 186° to 180° C., and by treating it with benzaldehyd mannose can be recovered, crystallising as rhombic crystals out of 90 per cent. alcohol. A 2 per cent. solution of mannose in water is dextro-rotatory ($a_D = +14\cdot25°$).

D-galactose does not occur free naturally. With dextrose, it may be prepared from the disaccharide lactose, or milk-sugar, by the action of dilute acids or enzymes. It also occurs in the trisaccharide raffinose, in combination with sucrose, in many gums and sea-weeds as the polymeric form galactan, and in the glucoside cerebrin of the brain spermatoza, pus, spleen, &c. From the brain it was isolated and described under the name " cerebrose " by Thudichum.

Galactose crystallises out of alcohol in thin, brittle, six-sided water-free plates, but from water in large rhombic prisms, or flat bright needles, containing one molecule of water of crystallisation. It is almost insoluble in absolute alcohol and ether. It is not so soluble in water as dextrose, and its solution is less sweet than cane-sugar. Solutions of galactose are more strongly dextro-rotatory ($+81°$) than dextrose. Fresh solutions show mutarotation. In chemical properties it resembles dextrose. It is fermented by some yeast, but not by all that ferment glucose. It reduces alkaline solutions of the heavy metals. A 1 per cent. solution of galactose reduces 4·7 molecules of copper oxide from Fehling's solution. It is only incompletely precipitated from its solutions by ammoniacal lead subacetate. With phenylhydrazin it forms a yellow osazone, consisting of stout needles which are slightly soluble in hot water and alcohol. The pure osazone melts at 196° C., but a melting-point of 193° to 194° C. is usually obtained. The acetic acid solution, unlike that of dextrosazone, is optically inactive. A pyridin-alcohol solution is slightly dextro-rotatory ($+0°$ 48'). With di-phenylhydrazin it forms a crystalline compound which melts at 157° C. It is, however, best recognised, and separated from other sugars, by the formation of the methyl-phenylhydrazone (melting-point 180° C.). Galactose is the only hexose which yields mucic acid on oxidation with dilute nitric acid, and by this property it may be recognised in a mixture of carbohydrates. With phloroglucin and hydrochloric acid (Seliwanoff's test) galactose gives a red coloration, but no absorption bands (*cf.* pentoses).

Laiose $(CH_2O)_n$.—Leo's sugar is a levo-rotatory golden syrup ($a_D = +26°$ 07'), which has been separated from the urine in diabetes. It has a saltish, but not a sweet taste, is not fermented by yeast, either before or after hydrolysis, reduces Fehling's solution, but more feebly than dextrose, gives no reaction with Nylander's solution, and forms with phenylhydrazin an oily compound that is insoluble in water, but is soluble in alcohol. With caustic alkalies it gives, on heating, a yellow, but not a brown coloration. It is not precipitated by lead acetate, but separates out on adding basic lead acetate to the solution,

and making it alkaline with ammonia. This precipitate does not change colour on heating. Unlike glucose it is not precipitated out of a methyl-alcohol solution by a methyl-alcohol solution of baryta.

Di-Saccharides.—*Maltose* ($C_{12}H_{22}O_{11}$) is prepared by acting upon starch with diastase, a ferment occurring in malt, saliva, and pancreatic juice, the only other product of the change being dextrin. It also appears as an intermediate product in the action of sulphuric acid on starch.

The amorphous anhydride is very hygroscopic, but it usually occurs in fine crystalline needles as the hydrate ($C_{12}H_{22}O_{11}+H_2O$). It is readily soluble in water and alcohol, but is insoluble in ether. Solutions of maltose are strongly dextro-rotatory ($a_D = +138°$), and exhibit upward mutarotation. Maltose is hydrolised to two molecules of dextrose when heated with dilute acids, but is far more resistant than cane-sugar. It is hydrolysed more rapidly by the enzyme maltase contained in many yeasts, and it is only a yeast containing this enzyme that is able to ferment it, since it is necessary that it should be converted into dextrose before the yeast can break it down into carbon dioxide and alcohol. The ferments diastase, invertase, lactase, and emulsin are without action on it. Maltase, prepared by extracting the dried yeast with water, affords an absolute means of identifying maltose. Maltose resembles dextrose in its power of reducing hot Fehling's solution without previous inversion, but the amount of cuprous oxide precipitated is only 62 per cent. of that reduced by an equal weight of dextrose. Unlike dextrose, it does not reduce a copper acetate solution (Barfoed's reagent), unless the boiling is prolonged, when hydrolysis of the disaccharide occurs, and reduction follows. Nylander's solution is reduced by maltose as by dextrose. On being heated with phenylhydrazin at the temperature of the water-bath, maltose forms an osazone which is, however, only precipitated out on cooling, and after an hour's heating. The osazones of dextrose and levulose are precipitated out after ten minutes' heating. Maltosazone is soluble in about 75 parts of hot water, whereas glucosazone is almost insoluble. Maltosazone is readily soluble in hot alcohol, and also in a cold mixture of equal parts of water and acetone. These properties of the osazones afford means of separating the sugars when they occur in a mixture, but before testing their solubilities it is essential that the osazones should be thoroughly washed with water and benzene to remove products which tend to make dextrosazone appear soluble. The osazone of maltose is not easily purified, and does not show a sharp melting-point, as it tends to decompose as this is reached. The melting-point is usually stated to be 205° to 206° C. Microscopical examination of the crystalline osazone shows yellow plates, or needles, which are usually broader and shorter than those of dextrose, but both the melting-point and crystalline form are greatly altered by small quantities of impurities. Theoretically the osazone should yield about 10 per cent. of nitrogen. On being oxidised with bromine, maltose

yields an acid with the same number of carbon atoms, which is hydrolised to glucose and gluconic acid by mineral acids.

Isomaltose was the name given by Fischer to a disaccharide obtained by treating a concentrated solution of dextrose with strong acids at a low temperature. It was separated out as an osazone with a melting-point of 150° to 153° C., and was found to be more easily soluble in hot water than maltosazone, 1 part in 4 as compared with 1 part in 75. Products similar to isomaltose have been repeatedly described as obtained in the hydrolysis of starch, along with maltose, by the action of diastase, ptyalin, amylopsin, &c., and an amyloptic ferment in the blood is said to have the same, or a similar, action, but definite proof of its presence in such cases is lacking. Small quantities have also been stated to be present in normal blood and urine. Isomaltose is probably identical with the disaccharide obtained by Crofton Hill through the action of maltase on glucose which he termed " revertose."

Isomaltose is said to be readily soluble in water, to be insoluble in alcohol and ether, and to have a very sweet taste. It reduces Fehling's and Nylander's solutions, having about four-ninths the reducing power of dextrose. It is not directly fermented by yeast, but undergoes slow changes. It is hydrolised by emulsin, but not by maltase or invertase. With a-naphthol and hydrochloric acid it gives a marked furfurol reaction. Its solutions are dextro-rotatory $(a_D = +139°$ to $149°)$.

Lactose (*milk-sugar*) occurs in the milk of all animals, and is occasionally met with in the urine. It has not been found in the vegetable kingdom. It is manufactured by evaporating whey, and is obtained as a white crystalline powder. It has a faint sweet taste, and is less soluble than other sugars (1 : 6 cold water, and 1 : 2 hot). It is insoluble in ether and absolute alcohol. Its solutions in water are dextro-rotatory $(a_D = +52° 7')$ and exhibit mutarotation. It rapidly reduces ammoniacal solutions of silver nitrate. Its reducing power for Fehling's solution is intermediate between that of dextrose and maltose, being roughly half that of dextrose. With phenylhydrazin it forms an osazone, which is soluble in 80 to 90 parts of hot water, and separates out on cooling, as aggregates of yellow needles with a melting-point of 200° C., and a nitrogen content of 10·76 per cent. Lactosazone, like maltosazone, is difficult to purify, and does not show a sharp melting-point as it decomposes on heating. Its crystalline form and melting-point are also materially altered by small quantities of impurities. Owing to the great solubility of the osazone small quantities of the sugar, such as occur in the urines of nursing women, cannot be detected by the phenylhydrazin test. Mineral acids hydrolise lactose to glucose and galactose, but it is less readily hydrolised than cane-sugar, being unaffected by being boiled for ten minutes with 2 grams of citric acid per 100 c.c. of the solution. Lactose is hydrolised by a specific ferment, " lactase," found in a few yeasts and in some kefir preparations, but not by maltase, invertase, diastase, nor

by any of the ferments of dried brewer's yeast. Lactase is also found in the secretions of the intestinal mucous membrane, particularly of the new-born. Lactose is particularly liable to undergo lactic and butyric acid fermentation. Heated with hydrochloric acid and phloroglucin, it gives a precipitate soluble in alcohol to form a red solution like a pentose, but does not show any bands on spectroscopic examination.

Isolactose is the name given to a disaccharide obtained by Fischer and Armstrong by the action of the enzyme kefir lactase on a concentrated solution of glucose and galactose, which they isolated in the form of an osazone.

Sucrose, saccharose, or cane-sugar is widely distributed in the vegetable kingdom, where it acts as a store of reserve material.

It crystallises well, forming large transparent colourless monoclinic prisms, known as sugar crystals and sugar candy. It is very soluble in water, forming a sweet, viscid liquid. It is much sweeter than dextrose, but is not as sweet as invert sugar. Cane-sugar dissolves in about half its weight of cold water, and is soluble in boiling water in all proportions. It is almost insoluble in absolute alcohol. In aqueous alcohol its solubility increases with the proportion of water. When subjected to prolonged boiling, solutions of cane-sugar acquire an acid reaction and are in part inverted. The dry substance when cautiously heated melts at about 160° C., and, on cooling, forms a transparent amber-coloured mass known as " barley-sugar." Above 160° C. it decomposes, turning brown, and forming so-called " caramel." On being heated with dilute mineral acids, cane-sugar is hydrolised to dextrose and levulose. A solution of cane-sugar is dextro-rotatory $(a_D = +66° 5')$, but does not exhibit mutarotation. Since fructose is more levo-rotatory than glucose is dextro-rotatory, the products of hydrolysis rotate polarised light in the opposite way to cane-sugar. The process of hydrolysis is hence termed " inversion," and the product " invert-sugar." A similar change is brought about by an enzyme present in yeasts, moulds, and in many plants, termed " invertase or sucrase." Cane-sugar is only fermented by yeasts after it has been previously inverted by the invertase of the yeast, and accordingly it is not fermented by yeasts that do not contain this ferment (*e.g. S. octosporus*). Cane-sugar lacks both aldehydic and ketonic properties. It does not therefore reduce Fehling's solution, does not form compounds with phenylhydrazin, and is stable toward alkalies. On treatment with moderately concentrated nitric acid it forms saccharic and oxalic acids. Cane-sugar forms definite compounds with some metallic oxides. Lead is attacked by sugar solutions slowly in the cold, but more quickly at boiling-point, the lead passing into solution. Calcium sucrate has an alkaline and bitter taste, and forms the liquor calcis saccharatus of pharmacy. Barium hydroxide forms a crystalline compound, which, on recrystallisation from boiling water, forms brilliant scales resembling boracic acid. It is only sparingly soluble in cold water. By means of strontium hydroxide, almost the whole

of the sugar may be separated from a solution as a granular precipitate. Crystalline compounds are also easily obtained with some sodium salts ; thus sodium chloride and iodide both enter into such combinations. These properties are made use of in the commercial separation and purification of cane-sugar.

Polysaccharides.—*Starch* $(C_6H_{10}O_5)_n$ is a characteristic product of the vegetable kingdom, and is found in almost every part of all plants.

It is a non-crystalline, colourless powder, composed of granules, which have a characteristic appearance under the microscope, and particularly under polarised light. The starch granule consists chiefly of a body, called granulose, which is coloured blue by iodine, together with a closely allied substance known as starch-cellulose, which gives only a dirty yellow colour with iodine. Starch-cellulose occurs in largest proportions in the outer layers of the granule, and probably constitutes the whole of the external covering. It is owing to the presence of this covering, which though slightly soluble is highly colloidal, that starch is unacted on by cold water. By boiling with water, starch-cellulose is mostly converted into soluble starch, and the granulose is allowed to escape. Iodine solutions readily permeate the outer layer of starch-cellulose, and colour the contained granulose of the solid starch an intense blue. Starch is insoluble in cold water, in alcohol, and in ether, but gelatinises and dissolves in hot water to form an opalescent solution. This solution is strongly dextro-rotatory $(a_D = +200°)$. The starch is precipitated out by ammonium sulphate, magnesium sulphate, ammoniacal lead acetate, and tannin. It does not reduce Fehling's solution, Moore's test is negative, it does not form an osazone with phenylhydrazin, and is not fermented by yeast. Starch is insoluble in Schweitzer's solution (ammoniacal cupric oxide). Heated with dilute acids it is converted into a mixture of dextrins and maltose, which ultimately yield the monosaccharide dextrose. Among the intermediate products is erythro-dextrin, which is apparently the first to develop. Achroo-dextrin appears later, and from this maltose, and finally dextrose, are formed. During the decomposition other dextrins of lower molecular weight are simultaneously produced, and these also yield maltose and dextrose, but finally one dextrin, termed malto-dextrin, is obtained which undergoes no further change. Similar changes are produced by the ferments diastase (amylase), pytalin, and amylopsin. The first step is the formation of amylo-dextrin, or soluble starch. This is decomposed into erythro-dextrin and maltose. In the third stage the erythro-dextrin is split up into achroo-dextrin and a further quantity of maltose. Finally part of the achroo-dextrin yields maltose, and part remains as a variety of dextrin not affected by the ferment. The action of acids is, however, more rapid, and is carried a stage further—namely, to dextrose. The ferment " maltase " found in the intestinal mucous membrane, and to a certain extent in some plants, has the power of converting maltose into dextrose.

Inulin $(C_5H_{10}O_5)_n$ is a substance akin to starch, found in the roots of various plants of the compositæ group, particularly in dahlias, dandelions, and chicory. It is the only polysaccharide that can be obtained in a crystalline form. It is met with as a white hygroscopic powder, or as sphæro-crystals. It is slightly soluble in cold water, and is readily soluble in hot water. On cooling the solution it is precipitated. Its solution is levo-rotatory. It is insoluble in absolute alcohol, and sparingly soluble in dilute alcohol. On being boiled with dilute acids it is converted into levulose. It reduces ammoniacal solutions of silver nitrate, but does not reduce Fehling's solution. It does not give any colour reaction with iodine.

Glycogen $(C_6H_{10}O_5)_n$ is as important a substance in the animal economy as starch is in plant life, forming the chief reservoir of carbohydrate material. It is present in the bodies of many protozoa, and is constantly met with throughout the animal kingdom, from the worms upwards. It exists as a small, but constant, constituent of the protoplasm of all animal tissues. In the lower forms it is found uniformly distributed throughout the cells (*e.g.* tænia 1·5 to 4·7 per cent., ascaris 4·2 to 7·1 per cent.), but in the higher animals it is more abundantly present in certain situations, notably the muscular tissues and certain portions of the alimentary tract (*e.g.* the mid-gut of molluscs and crustaceans) than in others. After the appearance of the liver, this organ becomes one of the chief seats of the deposition of glycogen. The quantity present in the liver is primarily dependent upon the state of nutrition of the animal and the amount of exercise that is taken. The maximal amounts, according to Külz, are found 14 to 16 hours after food. It has been calculated that in the liver of a man 150 grams can be stored at one time ; this would correspond to about 10 per cent. of an organ weighing 1500 grams. The quantity of glycogen which is deposited in the muscular tissues probably represents about half the total amount that is present in the entire body, and in man corresponds to about 150 grams. Occasionally traces of glycogen are met with in diabetic urines.

Pure glycogen is a white amorphous powder, which is both odourless and tasteless. It is slowly soluble in water, forming an opalescent fluid, which can be cleared by the addition of acetic acid. Pure solutions are strongly dextro-rotatory $(a_D = +197°)$. It is insoluble in alcohol and ether, and can be precipitated from watery solutions by the addition of alcohol, the precipitation being promoted by the addition of a little sodium chloride. It is also precipitated by baryta water, and, unlike dextrin, by basic lead acetate. Filtration through animal charcoal removes it from watery solutions. With benzoyl chloride, in the presence of sodium hydrate, it gives a granular precipitate of benzoyl-glycogen. It is readily soluble in alkalies. Glycogen does not reduce Fehling's solution, but can maintain cupric hydroxide in solution. After the addition of a little sodium chloride, its solutions are coloured red with iodine. On boiling with dilute acids it is transformed into dextrose. The ferments diastase and maltase produce

changes very similar to those induced in starch, a great part of the glycogen being converted into dextrose, and a small part into achroo-dextrin, with, in some instances, a small amount of maltose. It is not fermented by yeast. Glycogen does not form an osazone with phenylhydrazin.

Dextrin $(C_6H_{10}O_5)_n$ is the name given to a number of intermediate products formed during the hydrolysis of starch by dilute acids and diastase. The principal varieties are erythro-dextrin, which gives a red colour with iodine; achroo-dextrin, which gives no colour reaction with iodine; and malto-dextrin.

Dextrin is a white amorphous, tasteless, odourless, very delaquescent powder. It is readily soluble in water, and its solutions are strongly dextro-rotatory. It is insoluble in alcohol and ether. The dextrins do not reduce alkaline solutions of copper, but give a blue solution with Trommer's test. On being boiled for thirty minutes with dilute sulphuric acid, maltose, dextrose, and other reducing substances are formed, and these, after neutralising the acid with a sodium hydrate, reduce Fehling's solution. The dextrins are not coagulated by heat or mineral acids, and are not precipitated by tannin or baryta water. Heated with nitric acid they give rise to oxalic acid.

Cellulose is a characteristic product of the vegetable kingdom, forming the essential part of the solid framework of all plants. It is insoluble in water and all simple solvents. It is not hydrolised by boiling with dilute acids, but treatment with strong sulphuric acid, followed by dilution, gives rise to dextrose and other substances. It does not react with iodine solutions, but is turned blue after preliminary treatment with zinc chloride. It is soluble in Schweitzer's solution (ammon. cupric oxide), forming a levo-rotatory viscid solution. Cellulose is not acted on by the digestive ferments of the alimentary canal, but may be decomposed by intestinal bacteria into carbon dioxide and methane.

Gums.—Although gums are usually classed with carbohydrates, it has now been shown that they are really glucoside derivatives of certain organic acids. The acid is different in different gums, and is to be regarded as the nucleus of the particular gum. The commonest sugars in gums are galactose and arabinose.

The gums are a peculiar class of bodies occurring in the juices of plants. They are non-volatile, have little or no taste, and are uncrystallisable and eminently colloidal. They are either soluble, or swell up, in contact with water, giving a levo-rotatory solution. They are insoluble in alcohol, are not fermented by yeast, and do not react with iodine. On boiling with dilute acids they yield the pentoses or hexoses that are united to their acid nucleus. On treatment with moderately concentrated nitric acid they yield mucic acid. Solutions of gums give a gelatinous precipitate with copper and iron salts.

Animal Gum.—The substance separated, and first described by Landwehr, under the name of animal gum, is a decomposition product of mucin, and is probably not a chemical entity, but a mixture of sub-

stances that is precipitated from the urine by alcohol. Chemically it is not related to the vegetable gums.

In water it gives an opalescent solution, out of which it is not precipitated by alcohol. It gives no colour reaction with iodine. It is not precipitated by lead acetate, but is precipitated on the addition of ammonia to the solution. Boiled with dilute sulphuric acid it yields a reducing, but unfermentable, sugar, which has been termed " gummose " ($C_6H_{12}O_6$). The reduction of both copper and bismuth is slow and incomplete. Treatment with nitric acid gives oxalic acid, and with hydrochloric acid levulinic acid, leucin, tyrosin, &c. Copper and iron salts give a gelatinous precipitate, like that given with the vegetable gums. Boiling with hydrochloric acid and α-naphthol gives a well-marked furfurol reaction similar to that yielded by the pentoses.

Inosite is not a carbohydrate, as was at one time supposed, but belongs to the aromatic series, and is commonly regarded as hexahydroxybenzol. It is apparently a constant constituent of muscle, but is also found in other tissues of the body. It is not, however, peculiar to the animal world, but is also widely distributed in the vegetable kingdom. Inosite occurs in the urine in some cases of diabetes and albuminuria, and is met with when polyuria is artificially produced, or results from morbid processes. According to Hoppe-Seyler traces are present in all urines.

In the pure crystalline form it appears as colourless prisms which are often grouped in rosettes. It melts at 217° C. It has a sweet taste, but gives none of the characteristic reactions of the sugars. It is soluble in water and dilute alcohol, but is insoluble in absolute alcohol and ether. Inosite does not reduce the metallic oxides in alkaline solution, is optically inactive, and is not fermented by ordinary yeast. Bacterium lactis decomposes it with the formation of lactic acid, and it subsequently yields butyric acid.

The Carbohydrate Constituent of Proteins. — Many albumens when examined with the Molisch-Udransky reaction, or Bial's modification of the orcin test, give results which indicate the presence of a carbohydrate group in the molecule. Investigation of their degradation products has confirmed this, and a substance having the reactions of a carbohydrate has been separated from most proteins.

Such carbohydrate complexes are most readily separated from the glyco-proteins (mucins, cartilages, &c.), and can be prepared by simple hydrolysis, a fact which distinguishes this group of substances from the ordinary albumens, from which the sugar group, or the parent substance of the sugar group, is only eliminated by more drastic measures. The carbohydrate is usually obtained in the form of chitosamine or glycosamine, an amine or nitrogenous sugar ($C_6H_{11}O_5.NH_2$). Glucosamine does not exist preformed in the albumen molecule, but is a degradation product derived from the carbohydrate constituent of the molecule, which probably exists in the form of a polysaccharide (Fränkel's " albamin ").

Glucosamine-like complexes are not the only carbohydrate groups that may be derived from albumens, for it has been shown that, by the hydrolysis of certain nucleic acids, substances having the reactions of a pentose can be isolated. Thus from the nucleo-proteid of the pancreas, liver, &c., a carbohydrate identified as l-xylose has been prepared. It is not known whether all nucleic acids yield pentoses on hydrolysis, and it is probable that hexoses can be obtained from some.

The amount of reducing substance that can be separated from different albumens differs considerably. The largest proportion is yielded by the glyco-proteids, some 30 to 40 per cent., while crystalline egg-albumen gives from 10 to 11 per cent. Other albumens show a much smaller yield (*e.g.* serum albumen 0·5 per cent.). Casein appears to be the only animal albumen which does not yield a carbohydrate complex on hydrolysis, and the vegetable albumen appear frequently to lack a carbohydrate group.

The tend of all modern research has been to show that carbohydrate groups form no inconsiderable part of the whole albumen molecule, but much yet remains to be done before our knowledge of the subject can be considered satisfactory.

ACIDS AND ACID-DERIVATIVES OF THE SUGAR GROUP

Mono-basic Acids of the Sugar Group

Glycollic acid.	Glyceric acid.	Tri-oxybutyric acid.	Tetra-oxyvaleric acid.	Gluconic, Mannonic, Galactonic acids.
$CH_2(OH.COOH$	$C_2H_3'OH)_2.COOH$	$C_3H(OH)_3.COOH$	$C_4H_5(OH)_4.COOH$	$C_5H_6(OH)_5.COOH$
$CH_2.OH$ $\|$ $COOH$	$CH_2.OH$ $\|$ $CH.OH$ $\|$ $COOH$	$CH_2.OH$ $\|$ $(CH.OH)_2$ $\|$ $COOH$	$CH_2.OH$ $\|$ $(CH.OH)_3$ $\|$ $COOH$	$CH_2.OH$ $\|$ $(CH.OH)_4$ $\|$ $COOH$

The monobasic acids result from the action of feeble oxydising agents, such as bromine or dilute nitric acid, on the sugars. On evaporating their solution they are partly converted into a lactone, or intramolecular anhydride. The lactones are mostly less soluble, and more readily crystallisable, than the corresponding acids. They form characteristic compounds with strychnine, brucine, zinc salts, and with phenylhydrazin. If a 10 per cent. solution of a lactone is heated with a large excess of phenylhydrazin, and an equal quantity of 50 per cent. acetic acid, on a water-bath for half an hour, a hydrazide of the acid is formed. By heating this with baryta water the acid can be recovered. Glycollic and glyceric acids do not, however, give a compound with phenylhydrazin.

The most important members of this group are :—

Glycollic acid (*hydroxy-acetic acid*) is found in unripe grapes, and in the leaves of the wild vine. It can be prepared from dextrose by

oxidation with silver oxide. It appears as colourless needles, or plates, which melt at 80° C. It is hygroscopic, and is readily soluble in water, alcohol, and ether. Oxidised with nitric acid it yields oxalic acid.

Glycocoll (amido-acetic acid, $CH_2.NH_2.COOH$) is a constituent of the glycocholic acid of the bile. It is generally prepared by treating glue with acids or alkalies. It occurs as colourless prisms, soluble in water, insoluble in absolute alcohol and in ether. It has a sweet taste, and is consequently known as gelatine sugar or glycocoll.

Tri-oxybutyric acid is prepared from erythrose, or from levulose, by treating them with barium hydroxide and mercuric oxide.

Gluconic Acid (*Penta-oxycaproic Acid*).—On treating dextrose with bromine water and silver oxide, the aldehyde group is oxidised to carboxyl, yielding gluconic acid :—

$$
\begin{array}{ll}
\text{C.HO} & \text{COOH} \\
| & | \\
(\text{CH.OH})_4 & (\text{CH.OH})_4 \\
| & | \\
\text{CH}_2.\text{OH} & \text{CH}_2.\text{OH}
\end{array}
$$
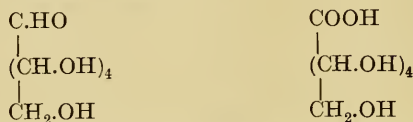
Mannose, galactose, and other aldoses, with their derived disaccharides, on being similarly treated yield acids corresponding to gluconic acid, known as mannonic, galactonic acids, &c.

Gluconic acid in solution readily passes into the anhydride or lactone form, a change which is accompanied by an alteration in the rotatory power of the solution. When heated with quinoline or pyridine, gluconic acid is partly converted into mannonic acid, a reaction which is reversible. This property has proved of much value in the synthesis of the sugars. Similarly galactonic and talonic acids are mutually interchangeable. The bacterium xylinum, or sorbose bacterium, oxidises aldoses to the corresponding monobasic acids, converting glucose into gluconic acid, galactose into galactonic acid, and the pentoses, xylose and arabinose, into xylonic and arabonic acids respectively. In all these cases the – C.HO group of the sugar is oxidised to – COOH, through the agency of the organism.

Lactic acid (*oxy-propionic acid*, $CH_3.CH(OH).COOH$) is the next highest homologue of glycollic acid. It is an oxyacid, and, since it contains an asymetrical carbon atom, can exist in a dextro-rotatory, a levo-rotatory, and an optically inactive form.

Inactive lactic acid (a-oxy-propionic, ethylidene, or fermentation lactic acid) is formed in the lactic acid fermentation of sugars, and substances related to them, by lactic acid organisms if the solution is nearly neutral, and also by the action of dilute alkalies on carbohydrates. It is a thick, syrupy liquid, which on being distilled *in vacuo* and strongly cooled separates out in a crystalline form. The crystals melt at 1° C., and are very hygroscopic and delaquescent. The acid is miscible with water, alcohol, and ether. It has a strongly acid taste and reaction. When heated, it is partly converted into the anhydride, lactide, and partly broken up into aldehyde, carbon monoxide, and water. On oxidation it gives acetic and carbonic acids. It may be

separated, purified, and recognised by the formation of the calcium, zinc, or strychnine salts. The calcium salt forms warty masses of microscopic needles, that are easily soluble in hot water, but are much less soluble in cold water (1 : 9·5), and are insoluble in cold alcohol. The strychnine salt is also a comparatively insoluble compound, and serves to separate it from the dextro-rotatory acid.

Dextro-rotatory lactic acid (sarco- or para-lactic acid) occurs in muscle, brain, &c., and, under pathological conditions, in the urine. It forms a colourless, odourless syrup which is easily soluble in water, alcohol, and ether. It is dextro-rotatory ($a_D = + 3\cdot5°$), but its salts are levo-rotatory. The most characteristic are the zinc and calcium compounds, which differ from those of the inactive acid in crystallising out with one molecule less of water, and in the zinc salt being much more easily soluble and the calcium salt being much more insoluble. The strychnine salt is also more soluble than that of the inactive acid. Like the inactive acid, it gives Uffelmann's reaction, a canary yellow colour with a 2 to 4 per cent. solution of carbolic acid and a drop of perchloride of iron solution. When heated it is converted into the lactide and aldehyde.

Butyric acid ($CH_3.CH_2.CH_2.COOH$) is produced in the fermentation of sugars and starches, but at a later stage than lactic acid. It also results when albumens are oxidised with chromic acid, and fats with nitric acid. It occurs free in perspiration, the juice of flesh, the contents of the large intestine, and in the fæces. It is met with in butter as a glycerine ester to the extent of about 2 per cent. Butyric acid is a thick, syrupy liquid with a rancid odour, miscible with water, but separating out from the watery solution on the addition of salts. It is only oxidised with difficulty in the laboratory. With calcium it forms a salt, which separates out as glancing plates, and which is remarkable in being more soluble in cold than in hot water. It is therefore deposited on warming the concentrated aqueous solution.

Beta-oxybutyric acid ($CH_3.CH(OH).CH_2.COOH$) occurs in the urine in severe cases of diabetes, scurvy, severe infectious conditions (*e.g.* scarlet fever and measles), and in starving insane persons, &c. It also appears in the urine in health after several days on a purely protein diet.

With water it forms a colourless syrup, but may be crystallised out as transparent plates with a melting-point of 49° to 50° C. The crystals are soluble in water, alcohol, and acetone. The fluid is levo-rotatory ($a_D = -24° 12'$). Its salts are also levo-rotatory. They are easily soluble in water, feebly soluble in absolute alcohol, and are precipitated from their solution by adding ether. On being heated with water, or dilute sulphuric acid, oxybutyric acid is converted into *a*-crotonic acid ($CH_3.CH : CH.COOH$) and water, the acid forming crystals with a melting-point of 71° to 72° C. By treatment with chromic acid it gives rise to acetone. The tests by which oxybutyric acid can be recognised in the urine and the methods of estimating it have been dealt with elsewhere (p. 104).

2 F

Aceto-acetic, or di-acetic, acid ($CH_3.CO.CH_2.COOH$) is so called because it may be considered to consist of two molecules of acetic acid ($CH_3.COOH$), minus one molecule of water.

Aceto-acetic acid does not occur in the urine of healthy individuals on a mixed diet, but is met with when under-nutrition and failure of absorption exist. It also occurs in healthy persons after some days on a purely protein diet. Pathologically it is met with in certain fevers, especially in children, in gastro-intestinal diseases, particularly in drunkards, and in severe cases of diabetes. It is only found when acetone is present, but not always then.

Aceto-acetic acid is a syrup that is easily soluble in water, alcohol, and ether. It is strongly acid in reaction. On being heated it is easily decomposed into acetone and carbon dioxide. Its salts are readily soluble in water. The tests and methods of estimating aceto-acetic acid are considered elsewhere (p. 104).

Acetone, or di-methylketone ($CH_3.CO.CH_3$), occurs in normal urine in small amounts, up to 10 mg. in the twenty-four hours. The output is increased when the carbohydrates of the food are limited, and the proteins are increased. Rich fat catabolism also increases the acetone in the urine, but it requires about 150 grams to produce any marked effect. Acetonuria is met with in febrile conditions, especially in children, in carcinoma in which inanition is not yet present, in states of inanition and cachexia, psychoses and lesions of the central nervous system, especially when associated with starvation, as a result of auto-intoxication, in digestive disturbances, particularly gastric ulcer, from chloroform narcosis, during pregnancy with a dead fœtus, and after certain poisons (*e.g.* phlorhidzin). Acetone is a colourless mobile fluid, with a pleasant fruity smell, that boils at 56° C. It is miscible with water, alcohol, and ether in all proportions. It can be separated out from its watery solution by the addition of salts, and particularly of calcium chloride. On being shaken with a concentrated watery solution of sodium bisulphite, it forms a colourless crystalline compound, that is readily soluble in water, and is quickly decomposed by dilute acids or alkalies, the acetone being regenerated. The estimation and tests for acetone in the urine are considered elsewhere (p. 104).

Di-basic Acids of the Sugar Group

Oxalic Acid.	Tartronic Acid.	Tartaric Acid.	Aposorbic Acid.	Saccharic, Mucic Acids.
$(COOH)_2$	$CH.OH.(COOH)_2$	$(CH.OH)_2(COOH)_2$	$(CH.OH)_3(COOH)_2$	$(CH.OH)_4(COOH)_2$
COOH | COOH	COOH | CH.OH | COOH	COOH | (CH.OH)_2 | COOH	COOH | (CH.OH)_3 | COOH	COOH | (CH.OH)_4 | COOH

By the action of energetic oxidising agents on the sugars both ends of the carbohydrate chain are oxidising, with the formation of di-basic

acids of the general formula COOH.(CH.OH)$_n$.COOH. Of these the most important are oxalic acid, and the isomers saccharic and mucic acids.

Oxalic acid is extensively produced in the physiological processes of plants, and to a less extent in animals. In plants it occurs as the free acid, or as sodium, potassium, or calcium salts, the last named forming crystalline deposits in the plant cells, known as " raphides." It is a normal constituent of the urine, the amount varying from 0·2 to 0·5 gram in the twenty-four hours. It is supposed to be present as the calcium salt, held in solution by di-acid sodium phosphate. It readily separates out on standing, and is then met with in the urinary sediment, and occasionally forms calculi. As certain articles of diet, such as asparagus, spinach, carrots, tomatoes, grapes, rhubarb, apples, plums, figs, strawberries, coffee, &c., contain a considerable amount of oxalic acid it is supposed that a certain proportion of that present in the urine is derived from the food, but as it does not entirely disappear on a diet of fat and protein, or even on starvation, a part must originate within the organism. From the close chemical relationship of oxalic to oxaluric acid, and of the latter to uric acid and the purin bodies, it is assumed that oxalic acid is formed from albumen, but the well-known tendency to increased oxalate excretion in diabetes, and the way in which a temporary diminution in the sugar output may be associated with an increase in the oxalates, have suggested that it may also arise from the incomplete oxidation of carbohydrates. According to Baldwin (*Journ. of Exp. Med.*, 1900), oxaluria may be caused by an excessive fermentation of carbohydrates. Oxalic acid may be prepared by the oxidation of sugar, starch, wood, and other organic bodies by the action of dilute nitric acid and other oxidising agents. It is made in bulk commercially by melting cellulose with caustic potash. It crystallises out with one molecule of water in the form of prisms. It is colourless, odourless, has an intensely sour taste, and an acid reaction. It is intensely poisonous. One part of oxalic acid is soluble in 10·46 parts of water at 14·5° C., and in 2·5 parts of cold alcohol, but is more easily soluble in hot alcohol ; 1·266 parts are soluble in 100 parts of ether at 15° C. It is insoluble in chloroform, benzene, and petroleum spirit. On heating it volatilises without charring at 150° to 160° C. With phenylhydrazin it forms glancing, colourless plates which soften at 170° C. The calcium salt of oxalic acid is insoluble in water, ammonia, acetic and other organic acids, but is soluble in dilute mineral acids (*e.g.* hydrochloric). It is re-precipitated on making the mineral acid solution alkaline with ammonia.

The Estimation of Oxalic Acid in Urine.—Treat 600 c.c. of fresh urine with a small quantity of an alcoholic solution of thymol, to prevent putrefaction. Make neutral, or faintly alkaline, with ammonia, and add an excess of a saturated solution of calcium chloride. The disodium phosphate which holds the oxalic acid in solution is thus removed. The precipitate is treated with just sufficient acetic acid to dissolve it. The calcium oxalate being insoluble in acetic acid, is gradually precipitated when the mixture

is allowed to stand for twenty-four hours. At the end of this time the calcium oxalate is filtered off, washed with a little water, and dissolved in dilute hydrochloric acid. Sufficient ammonia is added to the solution to give it a feebly alkaline reaction. After standing twenty-four hours the calcium oxalate will have separated out again. It is then filtered off, and treated in one of the following ways—(1) dried at 100° C., and weighed as calcium oxalate ; (2) ignited, moistened with ammonium carbonate, again gently ignited, and weighed as calcium carbonate ; (3) moistened on the filter with strong sulphuric acid and the whole ignited, again moistened with sulphuric acid, re-ignited, and finally weighed as calcium sulphate ; (4) it is ignited thoroughly and the resultant calcium oxide and carbonate weighed (56 parts = 128 Ca.Ox.) ; or better (5) titrated with standard acid ; or (6) the filter is placed in a beaker with water and dilute sulphuric acid, and the liquid titrated with standard potassium permanganate. The last two methods are probably the best, as they are least affected by impurity in the precipitate, but in the permanganate method the precipitate must be quite free from organic salts.

Blair Bell (*Brit. Med. Journ.*, 1912, i. p. 878) has described a method of estimating the calcium in the urine with the aid of the centrifuge, which he states gives accurate results.

Tartaric acid (di-oxy-succinic acid) occurs in some plant juices, but its only important source is grape juice. It is met with in four forms, physically isomeric :—

(1) *Dextro-rotatory, or ordinary, tartaric acid* is found in nature, and particularly as the acid potassium salt, especially in grapes. It forms large transparent prisms, easily soluble in water to form a dextro-rotatory solution. It is easily soluble in alcohol, but is almost insoluble in ether. It reduces ammoniacal solutions of silver on heating. Rochelle salt, used in the preparation of Fehling's solution, is potassium sodium tartrate $(C_4H_4O_6KNa)$.

(2) *Levo-rotatory form* is chemically identical with the dextro-rotatory form, but rotates the plane of polarised light in an equal and opposite direction.

(3) *Racemic tartaric acid* is a mixture of equal parts of the dextro- and levo-rotatory forms. It is interesting historically, as it originated the idea of isomerism.

(4) *Meso-tartaric acid* is optically inactive, like the above, but is not decomposable into active acids. It is produced by prolonged heating of the dextro-rotatory acid with a little water at 165° C.

Saccharic acid is produced by oxidising dextrose, and substances containing dextrose, such as dextrin, starch, &c., with nitric acid.

Saccharic acid is hydroscopic and easily soluble in water. It forms a sparingly soluble potassium salt, which serves for its separation.

Preparation of Saccharic Acid from Dextrose.—Two grams of dextrose are heated with 10 c.c. of nitric acid of a specific gravity of 1·2 (made by mixing 2 parts of Conc. HNO_3 and 1 part of water),

in a porcelain capsule on the water-bath, until a brisk reaction ensues and red vapours are given off. The heating is continued a few moments, and, when the reaction has subsided, the contents of the capsule are evaporated to a clear syrup to expel the excess of acid. Five or 6 c.c. of water are then added. The hot liquid is saturated with dry, powdered potassium carbonate, and 4 c.c. of glacial acetic acid are added. The mixture is well shaken, and cooled. The acid potassium saccharate, which is only slightly soluble, separates out as a white crystalline deposit. Microscopical examination of this shows that it consists of transparent needles free or arranged in rosettes.

Mucic acid is isomeric with saccharic acid, and is produced by the oxidation of galactose, and substances containing galactose, such as milk-sugar, gums, and mucilages, with nitric acid. It occurs as a sandy crystalline powder, which, unlike saccharic acid, is only feebly soluble in water (1 in 300 at $14°$ C.). It is insoluble in alcohol. It melts at $206°$ C., and at the same time undergoes decomposition. It is readily changed into furfurane (C_4H_4O).

Preparation of Mucic Acids from Galactose.—Two grams of the sugar are heated with nitric acid in exactly the same way as in the preparation of saccharic acid from dextrose, but when the oxidation is completed and the major part of the nitric acid has been evaporated off, 3 or 4 c.c. of water are added, and the contents of the capsule are poured into a test-tube. The capsule is then washed with water, and the washings added to the solution in the test-tube until a total of 10 c.c. is reached. A rapid precipitation of the mucic acid, in the form of a white powder, then takes place. This, on microscopical examination, is found to consist of small short prisms. The presence of mucic acid is confirmed by its complete solubility in ammonia, in contrast to the calcium oxalate formed on oxidising a mixture containing a calcium salt, which is insoluble in ammonia. Mixed with a few drops of ammonia, evaporated to dryness, and strongly ignited, mucic acid gives off pyrrol vapours, which colour a soft pine splinter soaked in concentrated hydrochloric acid a bright red colour.

The weight of the acid, collected on a weighed filter-paper, after standing for twenty-four hours, furnishes a means of approximately estimating the galactose in a mixture, 1 gram of sugar of milk under these conditions being equivalent to $0·33$ gram of mucic acid dried at $110°$ C.

Furfurol, furfurane aldehyde, or furfur aldehyde ($C_4H_3O.CHO$) is an aldehyde of pyro-mucic acid (furfurane carboxylic acid, $C_4H_3O.COOH$), which is formed by the dry distillation of mucic acid.

It is a colourless, oily liquid, with an agreeable odour resembling bitter almonds and cinnamon. It boils at $161°$ C., and turns brown on exposure to the air. It has the general properties of an aldehyde, and also shows characteristic colour reactions with certain substances by which it can be recognised. Its chief importance lies in the fact that it is produced on heating sugars, and substances containing a carbohydrate radicle, with hydrochloric, or sulphuric acid, of suitable strength. The readiness with which the sugars yield furfurol varies

with the nature of the sugar and the conditions under which the experiment is carried out, and on this is based a number of tests for the differentiation of those which, like the pentoses and ketoses, readily give much furfurol. With bodies of the phenol series it forms coloured compounds which vary in appearance with the nature of the sugar, and also with that of the phenol employed. Thus with orcin, in the presence of strong hydrochloric acid, the pentoses give a blue colour, or a green if iron is present, the methylpentoses and hexoses a red orange colour. With phloroglucin and concentrated hydrochloric acid the colour is red in all cases. The reaction of Seliwanoff, which distinguishes between aldoses and ketoses, is based upon the fact that the latter form furfurol when treated with hydrochloric acid diluted with its own bulk of water, whereas the former do not. The phenol employed in this case is resorcin, which gives a red colour. The reaction is most usually employed for the detection of levulose. Furfurol gives colour reactions with other substances, such as xylidine, amyl alcohol, aceto-acetic ether, acetone, brucine, a-naphthol, thymol, and aniline, all of which show a red colour. One of the most delicate of these, showing one part in a million, is aniline acetate. This reaction is particularly useful, as it is peculiar to furfurol.

If equal parts of pure aniline, glacial acetic acid, and water are boiled together for a few minutes, to destroy any furfurol that may be present in the acetic acid, 1 c.c. of the reagent cooled and added to 20 c.c. of a fluid containing furfurol shows a rose-pink colour in ten to fifteen minutes. Filter-paper moistened with the reagent held in the vapour arising from a boiling solution containing furfurol gives the same colour change.

Cholic acid and sulphuric acid show with 1 part in 20,000 of furfurol a crimson colour which forms the basis of Pettenkoffer's reaction for bile acids. With urea nitrate furfurol solutions give a violet coloration and deposit a black precipitate. Ammonium sulphide gives a yellow crystalline precipitate. With phenylhydrazin furfurol forms furfurol-phenylhydrazone (1 : 10,000). This is a crystalline body of a pale yellow colour and conspicuously pearly lustre, which is insoluble in water, ether, and cold alcohol, but is soluble in hot alcohol and in ether. The purified product melts at 97° to 98° C.

Glucuronic (*glycuronic*) *acid* ($CHO.(CH.OH)_4COOH$) is the only important representative of a series of oxidation products intermediate between the mono- and di-basic acids of the sugar group :—

$$
\begin{array}{ccc}
CH_2.OH & CHO & COOH \\
| & | & | \\
(CH.OH)_4 & (CH.OH)_4 & (CH.OH)_4 \\
| & | & | \\
COOH & COOH & COOH \\
\text{Gluconic acid.} & \text{Glucuronic acid.} & \text{Saccharic acid.}
\end{array}
$$
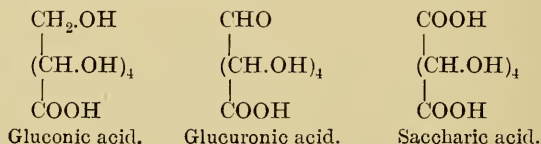
It is formed in the animal economy as a product of the metabolism of carbohydrates, being derived from dextrose by oxidation of the

primary alcohol group (CH_2OH) to carboxyl (COOH). It is therefore at once an aldehyde and an acid. It is met with in the shape of ether-like derivatives, combined with substances containing an hydroxyl group, in traces in the urine and blood. It has not yet been identified as a plant product.

Glucuronic acid may be prepared by reducing saccharic acid. On heating this substance on a water-bath for five or six hours saccharo-lactonic acid ($C_6H_8O_7$) is formed. If this is reduced by sodium amalgam, glucuronic acid results. Glucuronic acid is, however, most readily prepared from Indian yellow or Purre, the magnesium salt of euxanthic acid, obtained from the urine of cows fed on mango leaves, by hydrolysing it with dilute hydrochloric, or sulphuric, acid.

$$C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$$
Euxanthic	Euxan-	Glucuronic
acid.	thon.	acid.
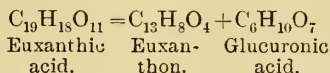
Glucuronic acid is a syrupy liquid, soluble in water and alcohol, but insoluble in ether. When its aqueous solution is boiled, evaporated, or even allowed to stand, it readily loses the elements of water and forms an anhydride or lactone ($C_6H_8O_6$).

The anhydride form crystallises in needles, or plates, which have a sweet taste, and melt at 167° C. It is insoluble in alcohol, but is readily soluble in water, forming a dextro-rotatory solution ($a_D = +19°\ 25'$). The solution prevents the precipitation of cupric salts by alkalies, and exerts a powerful reducing action on Fehling's solution when heated, and to a less extent in the cold. On being distilled with hydrochloric acid, glucuronic anhydride yields furfurol.

Glucuronic acid and its alkaline salts are dextro-rotatory ($a_D = +35°$). Most of its compounds are levo-rotatory. It is not fermented by yeast, but is slowly decomposed by bacteria under suitable conditions, yielding lactic and acetic acids. It reduces alkaline solutions of copper (98·8 parts of Fehling's solution as compared with 100 by dextrose), bismuth, mercury, and silver on heating, and when pure in the cold. Glucuronic acid is the only substance, other than the sugars, commonly occurring in the urine which reacts with phenyl-hydrazin. It forms a yellow crystalline compound resembling gluc-osazone, but the pure product has a melting-point of 114° to 115° C., as compared with 204° to 205° C. for glucosazone. The impure product, such as is obtained by treating urine with phenylhydrazin, does not crystallise readily, and melts at about 150° C. Theoretically the osazone should yield 18·4 per cent. of nitrogen, but it is difficult to obtain a sufficiently pure product to make the estimation reliable. With para-brom-phenylhydrazin, glucuronic acid forms a very characteristic light yellow, crystalline compound, which, owing to its feeble solubility, is most useful in separating glucuronic acid from the sugars. It is readily soluble in acetic acid, but is only very slightly soluble in hot water, benzol, ether, and absolute alcohol. The purified product melts at 236° C., but the impure material prepared from the urine

generally melts at 200° to 216° C. A solution of 0·2 gram dissolved in 4 grams of pyridine and 6 grams of alcohol is levo-rotatory ($a_D = -7° 25'$). Glucuronic acid may be set free from this compound by heating it with acetic acid. On oxidising glucuronic acid with bromine, it yields saccharic acid, and on being reduced with sodium amalgam it gives gluconic acid. When boiled with caustic alkalies glucuronic acid forms oxalic acid, catechol, and other products. Glucuronic acid forms potassium and sodium salts which crystallise in needles. The zinc, cadmium, copper, silver, and calcium salts are uncrystallisable. Treated with an excess of baryta water a solution of glucuronic acid, or one of its inorganic salts, yields an insoluble yellowish-white to orange-coloured precipitate of the basic barium salt, which can be employed for the separation of glucuronic acid from urine. With quinine, glucuronic acid forms an easily crystallisable salt which is useful in preparing the acid from its organic compounds, and separating it from the sugars. Lead acetate gives a white precipitate of the basic lead salt. Glucuronic acid is precipitated from acid solutions by basic lead acetate, in contrast to the sugars which only separate out in an alkaline medium. On being boiled with hydrochloric acid, glucuronic acid yields furfurol, and hence gives the phloroglucin and orcin tests like the pentoses. The furfurol is, however, produced more slowly, so that a higher temperature and more prolonged heating are necessary.

$$C_6H_8O_6 = C_5H_4O_2 + 2H_2O + CO_2$$

Glucuronic   Furfurol. Water.   Carbon
anhydride.   dioxide.
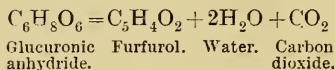
By combining the furfurol with phloroglucinol the glucuronic acid may be quantitatively estimated, 1 part of furfurol phloroglucide corresponding to 3 parts of glucuronic anhydride. Carbon dioxide is also liberated when glucuronic acid is treated with hydrochloric acid, and may be used to estimate it in the presence of pentoses, 1 part of carbon dioxide corresponding to 4 parts of glucuronic anhydride. Glucuronic acid can be distinguished from the pentoses by the blue substance, soluble in ether, formed when it is boiled with naphtho-resorcinol and hydrochloric acid (Tollens).

Combined, Paired, or Conjugate Glucuronic Acids.—When certain substances that contain an hydroxyl group, and are only oxidised with difficulty, are introduced into the body they combine with dextrose to form ether-like compounds. In these one end of the chain is shielded from attack by the pairing substance, but the other is open to chemical change. When this is oxidised glucuronic acid is formed, and the paired, or conjugate, glucuronate is excreted in the urine.

R.C.CH(CH.OH)$_2$.CH.CH(OH)COOH
Paired glucuronic acid.
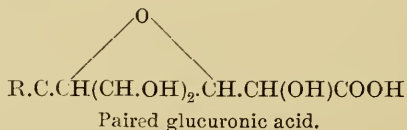
The number of substances thus excreted in the urine in combination with glucuronic acid is very large. The most important are the following :—

Isopropyl alcohol, methylpropyl carbinol, methylhexyl carbinol, tertiary butyl alcohol, tertiary amyl alcohol, pinacone ;

Chloral, butylchloral, bromal, dichloracetone ;

Benzene, nitrobenzene, aniline, phenol, resorcinol, thymol, a- and β-naphthol ;

Turpentine oil, camphor, borneol, menthol, pinene, antipyrin, &c.

Most of the compound glucuronates are of a glucosidal nature, resembling the glucosides met with in plants.

$$R.O.CH(CH.OH)_2.CH.CH.(OH).CH_2(OH)$$
Glucoside.
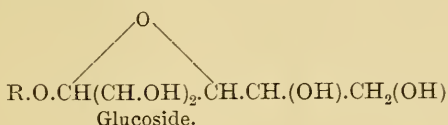
This is shown by the fact that, like the latter, they are attacked, and broken down, by appropriate glucoside-splitting ferments, and also that, like the glucosides, the conjugate glucuronates are hydrolised by mineral acids, yielding glucuronic acid and the particular alcohol from which they were formed. All conjugate glucuronic acids do not, however, exhibit the characters of glucosidal compounds, for some, such as urochloral acid and camphor-glucuronic acid, are capable of directly reducing Fehling's solution, a reaction which is only obtained with most conjugate glucuronic acids after the acid has been set free by hydrolysis. This property appears to depend upon freedom of the aldehyde group in the combined acid. Urochloral acid (trichlorethyl glucuronic acid) is excreted in the urine after large doses of chloral hydrate ($C.Cl_3.CHO$). On being heated with a mineral acid it yields trichlorethyl alcohol ($C.Cl_3CH_2.OH$) and glucuronic acid. In the same way camphor glucuronic acid appears in the urine after large doses of camphor ($C_{10}H_{16}O$), and on hydrolysis yields camphoral ($C_{10}H_{15}O.OH$) and glucuronic acid. In both cases the glucuronic acid is combined with an alcohol, which, in the one instance, has been derived by reduction, and, in the other, by oxidation within the body.

In addition to the non-nitrogenous conjugate glucuronic acids, a nitrogen-containing variety, uramido glucuronic acid, has been described. This on being heated with barium hydrate yields ammonia, carbon dioxide, and glucuronic acid free from nitrogen.

Most conjugate glucuronic acids, and their alkaline compounds, are easily soluble in water, and the former are also readily soluble in alcohol and ether. The potassium salts can be crystallised out from an alcohol-ether extract of the urine after being set free from its compounds. The glucuronates are as a rule readily precipitated by lead acetate, basic lead acetate, and by lead acetate and ammonia. The feebly soluble basic lead and barium salts can be used to separate glucuronic acid after it has been set free from its compounds, the acid

being subsequently recovered by treating the compounds formed with sulphuretted hydrogen, and sulphuric acid, respectively. A certain amount of the conjugate glucuronic acid may be recovered from urine, by shaking an acidified alcoholic extract of the hydrolised urine, with a mixture of equal parts of alcohol and ether. Alkaloids form crystalline compounds with most conjugate glucuronic acids.

The conjugate glucuronates are levo-rotatory, and the presence of small quantities in normal urine accounts for its slight levo-rotatory power ($a_D = -0.01°$ to $0.18°$). The alkaline salts of glucuronic acid are, however, dextro-rotatory, like the free acid. Conjugate glucuronates, like the acid itself, are not fermented by yeast. Many conjugate glucuronic acids do not reduce alkaline solutions of the heavy metals until they have been decomposed by heating with an acid and the glucuronic acid has been set free, but some, such as chloral, camphor, menthol, turpentine, and indoxyl compounds reduce Fehling's solution on simply boiling. The slight reducing power of normal urines is more or less due to the presence of glucuronic acid compounds. Normal urine also gives a reaction with phloroglucin and hydrochloric acid, and the orcin reaction, after it has been boiled with 1 per cent. sulphuric acid for one minute, for the same reason. The conjugate glucuronic acids do not form crystalline compounds with phenylhydrazin until the glucuronic acid has first been set free.

INDEX